Progress in Nutrition 2020; Vol. 22, N. 2: 00-00 DOI: 10.23751/pn.v22i2.8180

Original article

Levels of vitamin D and vitamin D pathway gene polymorphisms in adults: results based on rural agriculture workers in Punjab province of Pakistan

Nadia Hussain[1], Amira S.A Said[2], Mirza Rafi Baig[3]

1Department of Pharmaceutical Sciences, Faculty of Pharmacy, Al Ain University of Science and Technology, Al Ain, UAE - E-mail: nadia.hussain@aau.ac.ae; 2Department of Clinical Pharmacy, Faculty of Pharmacy, Al Ain University of Science and Technology, Al Ain, UAE; 3Clinical Pharmacy and Pharmacotherapeutics Department, Dubai Pharmacy College, P.O.Box: 19099 , Dubai, UAE

Summary. *Background and aims:* Worldwide low levels of vitamin D (< 30ng/dL) is prevalent and linked to bone disorders, diabetes and coronary heart disease. Deficiency in Vitamin D is a diagnostic challenge in asymptomatic individuals. The present study aimed to assess the environmental and also the genetic determinants affecting vitamin D deficiency in an asymptomatic rural agricultural population sample of Punjab province of Pakistan. *Material and method:* An interview based questionnaire and blood samples for measuring serum markers were obtained. These include calcium levels, parathyroid hormone and serum 25(OH) vitamin D. DNA was extracted from the blood samples for genotyping. *Results:* From 510 study participants, 435 (85.2%) individuals had < 30 ng/dL (low) of vitamin D. Males versus females had a unremarkable difference in the status of Vitamin D (61.3% vs 56.2%), (p = 0.134). When calcium levels were compared between the deficiency and insufficiency groups versus the vitamin D sufficiency group, no significant difference was observed (p = 0.526, 0.155 respectively). Consumption of single milk serving every day (250 ml) (p = 0.818) and sunlight exposure every day for more than 30 minutes (p = 0.579) also had non-significant associations with the estimated vitamin D levels. However, oral vitamin D supplementation was significantly associated (p = 0.024) with the vitamin D levels. Eight SNPs were studied and none showed any statistical significant association with observed vitamin D levels. *Conclusions:* We noted a considerable proportion of asymptomatic individuals from the rural population with low vitamin D levels. There appear to be multifactorial causes of deficiency in vitamin D and this burgeoning health issue requires further investigations.

Keywords: Vitamin D, nutrition, sunshine hours, agriculture workers, Punjab, Pakistan

Background and aims of study

Vitamin D (25 OHD) is a vital vitamin for the calcium homeostasis of the human body. The form Vitamin D3 is produced in sunlight exposed skin to fulfil human needs. Sufficient vitamin D levels are pivotal for bone development and maintenance. This is due to Vitamin D influencing the small intestine's role in absorbing calcium and also it works the parathyroid hormone to affect skeletal mineralization (1).

For both children and adults, serum levels > 30ng/ml are adequate and insufficiency is classes when vitamin D levels are in the range of 20-30ng/ml (2), while values lower than 20 ng/ml are classed as Vitamin D

deficiency. Worldwide insufficient vitamin D levels (< 30ng/ml) are prevalent and WHO estimates it affects more than one billion people across the globe (3). Vitamin D deficiency was initially considered unusual in parts of the world that has access to sunshine throughout the year. Vitamin D levels are influenced by the population being assessed, regional food fortification practices, geographic location and season (4).

Bone disorders such as osteoporosis, osteomalacia and rickets are linked to inadequate levels of 25OHD (5)]. Several epidemiologic studies have discovered relationships between inadequate vitamin D levels and multiple disease states (6) e.g., type 1 diabetes mellitus and coronary heart diseases which are common disease states (7). Vitamin D is an immune modulator, tumour suppressor and has influence over the function of several genes (8).

Vitamin D deficiency can be asymptomatic and this can make it a challenging diagnosis. In Pakistan, the prevalence of low levels of vitamin D has been identified as in the range 85–98% (9)]. This has included studies done in Pakistan and neighbouring south Asian countries such as India (10, 11). In Pakistan, there have been several studies on Vitamin D levels that have focused on outpatient settings and in single centers (3, 12). In our current awareness, this study is novel because it is probably an inaugural study of its type conducted in the province of Punjab on asymptomatic adults from the agriculture based rural population.

Exposure to sunlight is a topic of epidemiologic interest because of both its benefits and harmful effects on human health. Pakistan has plenty of sunshine throughout the year and the UV index is from 5 to 11 during the different seasons of the year (13). The rural population of agriculture driven Punjab province of Pakistan is exposed to more than adequate sunlight due to the nature of the work such as the involvement in agriculture, field work etc.

The individual status of vitamin D is also influenced by genes as well as environmental factors e.g., diet and sun exposure. Gene variations for the genes that encode for vitamin D 25-hydroxylase enzyme (CYP2R1) and the vitamin D receptor (VDR) have been associated with an increased risk of developing deficiency in vitamin D (14). In our awareness, the rural population of Punjab province in Pakistan has not been subject to a study of genetic determinants in addition to the environmental influences that affect deficiency in vitamin D.

Material and method

Ethics

The methodology of the study was approved by the Punjab Care Hospital ethical review committee (ERC protocol-158925). Formal informed consent was received from all study participants. The participants had their laboratory results sent to them using the local postal service.

Medical camps were established for two weeks in each village to obtain data from the study participants. Ethnic diversity along with a high population density and ethnic diversity made these sites fairly representative of the adult population of the rural population in Punjab. A pamphlet in the national language of Pakistan (Urdu) was distributed that described the importance of Vitamin D and its sources for literate people. For illiterate people volunteers explained the study and its importance. This informed the study participants concerning the study's research objectives.

Size of the study sample

The primary objective of this study focused on establishing the prevalence of deficiency in vitamin D in asymptomatic individuals in a rural setting. We used the NCSS PASS software (NCSS LLC, Kaysville, Utah) to calculate our sample size to be 170 people from each village (a total of 510 study participants). We used an anticipated population proportion of 85% because the deficiency in vitamin D prevalence in our target region is between 85–98%. We estimated that the prevalence of insufficient vitamin D levels would be within 5% of the true value with 95% confidence. The objective was to target healthy asymptomatic adults between the age 30 till 60 years. Exclusion criteria included people with signs and symptoms of hypocalcaemia, diagnosed metabolic bone disease and females that were either pregnant or breast feeding. The medical camp did not allow inclusion of individuals who were restricted to their homes because of co-morbidities and physical

disabilities. In this way less mobile, ill patients were not included in this study.

Data collection

At the camps, an interview based questionnaire was administered by two trained volunteers. Information about age, education level, annual income, the presence of co-morbidities (diabetes, ischemic heart disease and hypertension), consumption of daily milk, sun exposure (approximate minutes spent per day in the presence of direct sunlight) and medication use was recorded. This was followed up by a physical assessment including anthropometric measurements with a balance scale.

Blood collection

Every study participant underwent a 10 ml venous blood sample withdrawal that was added to three plastic serum tubes. Of the 10 ml, 7 ml was immediately placed in EDTA tubes and 3ml in serum vials for analysis. The lab received the samples as batches packed in ice boxes. Approximately 30 minutes was the time lag between collection and serum separation after centrifugation (3000bpm). The freezer in the laboratory was maintained at -20 °C and used to store the centrifuged samples till further analysis. The measured serum markers were; calcium (Ca), parathyroid hormone (PTH), and serum. We used Diasorin SRH kits for the radioimmunoassay technique to measure 25(OH)D. EDTA plasma collection tubes was used to collect venous blood samples for PTH analysis.

Using the protocol by Iranpur et al. we used whole blood for DNA extraction and then proceeded to quantify the DNA using a nanodrop spectrophotometer. We used pre-developed TaqMan allelic discrimination assays to run a 10 ng of DNA sample. The TaqMan assays were used to type polymorphisms in the genes encoding for; CYP2R1 (rs2060793, rs10500804 and rs10766197) and rs4588 [StyI], VDR (rs2228570 [FokI], rs1544410 [BsmI]), rs731236 [TaqI]. Alleles at all loci did conform to the Hardy-Weinberg equilibrium.

Statistical analysis

We used the Statistical Package for Social Sciences version 25 (SPSS v. 25.0.0.0 ®) to analyze all the data collected in the study.

We did not consider using ANOVA and linear regression tests due to the asymmetric distribution of the biochemical markers and the 25(OH)D outcome variable. Descriptive statistics for age, education and socio-economic status of the individuals that took part in the study. For the categorical variables we calculated proportion and percentages. To test the normality assumption for the continuous variables, we used the Shapiro Wilk test. We also used the inter-quartile range (IQR) and median values to describe the sample.

Since we planned to use bi-variate analysis, we divided the serum 25(OH)D levels into three categories. These categories were deficiency, insufficiency and sufficiency. The Chi square test identified links between the different 25(OH)D level groups and for other categorical variables. The p-value of ≤ 0.05 was considered to be statistically significant.

For identifying the genetic determinants of the status of vitamin D we used multivariate analysis to study polymorphisms in genes encoding for the vitamin D receptor and the vitamin D 25-hydroxylase enzyme; CYP2R1. Unpaired t tests analyzed the connections between the serum 25(OH)D values and the genotype.

Results

Basic characteristics of sample population:

The study participants were a total of 510 individuals with 65% of the respondents being male. The minimum age and maximum age range was 31 and 60 years respectively with a median age (years) of 49 (IQR 37-53). Annual median gross annual income was 2000 $ (interquartile range $1150-3402) and in terms of education 25% of the individual held a college degree, 25% having graduated high school and 50% had no formal education at all.

Table 1 details the sample characteristics of the groups; vitamin D sufficiency (n-75), insufficiency (n=180) and deficiency (n=255).

The level of serum 25(OH)D was median 16.8 (IQR 11.3–23.2) ng/ml. Sample ranges of serum 25(OH)D levels were from 6.21 - 46.22 ng/ml. There was a sum total of 430 (84.1%) individuals who had insufficient serum 25(OH)D levels (< 30 ng/ml), and

Table 1. Characteristics of the three categories of Vitamin D according to levels of 25(OH)D

Characteristics	Deficiency (n = 255)	Insufficiency (n = 180)	Sufficiency (n = 75)	P Value
Age (years)	49.1 (38.0–56.0)	48.2 (39.5–56.5)	52.0 (40.8–64.2)	0.021
Male (n=331)	183 (55.2%)	100 (30.4%)	48 (14.4%)	
Female (n=179)	111 (62.3%)	36 (19.8%)	32 (17.9%)	
Median serum 25(OH)D levels ng/ml	14.45 (9.9–17.1)	24.81 (21.6–25.6)	35.92 (32.9–41.8)	0.001
PTH (pg/ml)	38.4 (34.3–56.8)	36.8 (26.9–43.9)	35.2 (28.0–48.8)	0.001
Calcium (mg/dL)	8.46 (9.24–9.67)	9.44 (8.92–9.68)	9.29 (9.23–9.71)	0.189
Phosphorus (mg/ml)	3.24 (2.71–3.43)	3.02 (2.88–3.45)	3.28 (2.78–3.61)	0.846
Vitamin D supplement use	86 (16.6%)	32 (6.2%)	18 (25.7%)	0.023
Daily sunlight >30 mins (n=435)	322 (78.3%)	62 (15.2%)	26 (6.5%)	0.092
Levels of 25(OH) *Vitamin D*				

305 of these individuals (59.7%) belong to the group 25(OH)D deficiency.

When comparing the genders of the individuals, the serum 25(OH)D levels in males median level was 17.99 (IQR 11.4 – 23.5) ng/ml and this compared to 17.12 (IQR 13.6– 23.1) ng/ml in females. We found that 86% percent and 82% of females had 25(OH) vitamin D levels < 30 ng/ml that is the cut off value for vitamin D sufficiency.

Females had more deficiency in serum 25(OH) D when compared to males (61.3% vs 56.2%) but this difference was not significant (p = 0.134).
Figure 1 illustrates the sample distribution of serum 25(OH)D levels.

Parathyroid hormone levels

The range of PTH was found to be 12 - 152 pg/mL. We estimated a significant negative correlation to be between the parathryroid levels and 25(OH) D levels (r=-0.189, p = 0.001). In comparison to the 25(OH)D deficient group, the insufficient 25(OH)D group had a statistically significant difference in PTH levels (p = 0.012). This was not observed when comparing the insufficiency and sufficiency groups (p = 0.800).

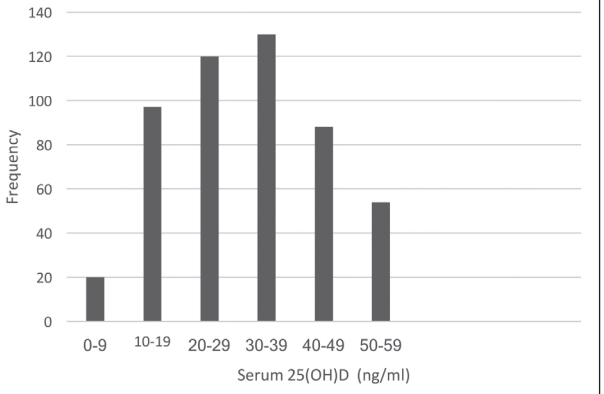
There was evidence of hyper-parathyroidism (PTH > 87 pg/mL) in 24 study participants (4.6%) having a 25(OH)D level of median 14.8 (IQR 8.78–25.87) ng/ml. Twelve of these identified study participants had a serum 25(OH)D level of median 12.85 (IQR 8.05–19.20) ng/ml which classed them as having deficient levels of vitamin D.

Calcium and phosphate levels

Non-significant difference was observed in calcium levels when the insufficiency and deficiency groups were compared to the 25(OH)D sufficiency group (p = 0.526, 0.155 respectively). The serum calcium level observed was median 8.46 (IQR 8.12–9.98) ng/dL in 61 (13.6%) of the study participants and serum phosphate levels < 2.5 mg/dL.
In 10 (1.3%) individuals, an increased phosphate level (>4.5 mg/dL) was estimated and they all had 25(OH) D levels of <30 ng/mL.

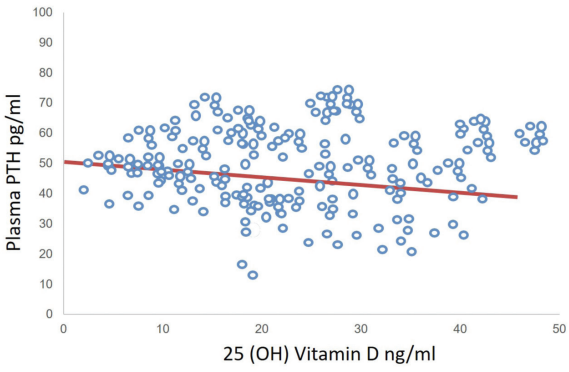
Supplementation with vitamin D

Levels of vitamin D were significantly associated with the consumption of oral vitamin D supplements



Distribution of serum 25(OH) vitamin D in blood samples of study participants. Frequency vs ng/ml – nanograms per milliliter.

Figure 1: Distribution of 25(OH)D levels found in the study sample



Scatter plot showing negative correlation between vitamin D and PTH. Plasma PTH (pg/ml) versus 25 (OH) Vitamin D (ng/ml). A significant negative correlation was estimated to be between the parathryroid levels and 25(OH) D levels (r=-0.189, p = 0.001).

Figure 1: Scatter plot showing negative correlation between vitamin D and PTH

every day (p = 0.024). A total of 137 (26.8%) of all study participants stated the use of vitamin D containing oral supplements with 58 (11.4%) individuals taking one dose every day. This amounted to 86 (16.6%) individuals using supplements containing vitamin D in the 25(OH)D deficiency group and 18 (25.7%) individuals in the sufficiency group. The type of supplement used by the study participants was the most commonly prescribed multivitamin in Pakistan (Centrum™) that contains 400 IU of Vitamin D.

Diet and sunshine in relation to Vitamin D

No significant association was found between daily sunlight exposure and consuming one daily milk serving to the 25(OH)D levels. The p values of these statements were 0.818 and 0.579, respectively.

Genotyping

We studied eight SNPs and none showed a statistically significant association with the levels of 25(OH) D. The results are summarized in Table 2.

Discussion

Our study sample involved asymptomatic adults of the rural community. This suggests that this health concern affects a sizeable majority of apparently healthy individuals. They are being denuded of the known benefits that vitamin D can provide. This is because we found insufficient levels of 25(OH) vitamin D (< 30 ng/ml) in 85.2% of study participants. Males and females were affected equally.

We found that 85.2% individuals had insufficient levels of 25(OH)D in the present study and is in accordance to prior studies conducted in other provinces such as Sindh. However, we did observe that 14.6% of individuals had sufficient levels of 25(OH)D (>30 ng/ml) compared to the results of 8.9% and 8.0% report-

Table 2. 25(OH) Vitamin D status influenced by genetic determinants in healthy adults (males and females)

SNP	Genotype	Serum 25(OH) D <50 nmol/L N (%)	[a]OR (95 % CI)	P value
VDR, rs731236[a]	AA	55/ 68 (81%)	Ref	-
	AG/GG	93/120 (77%)	1.57 (0.65–3.78)	0.45
VDR, rs1544410[b]	CC	80/99 (80%)	Ref	-
	CT/TT	72/89 (80 %)	1.78 (0.65–3.78)	0.29
VDR, rs2228570[c]	GG	39/49 (78 %)	Ref	-
	GA/AA	62/68 (76 %)	2.07 (0.84–5.09)	0.13
VDR, rs731236a[d]	GG	23/29 (78 %)	Ref	-
	GA/GA	42/58 (71 %)	0.93 (0.33–2.78)	0.64
VDR, rs731236a[e]	AA	31/39 (79 %)	Ref	-
	AG/GG	52/ 72 (71 %)	1.97 (0.95–3.01)	0.66
VDR, rs731236a[f]	AA	49/58 (83 %)	Ref	-
	AC/CC	68/89 (76 %)	2.51 (0.79–4.00)	0.06
CYP2R1, rs10500804[g]	GG	65/87 (74 %)	Ref	-
	GT/ TT	31/42 (72 %)	0.87 (0.65–3.78)	0.12

[a] OR odd ratios were multivitamin supplementation use, time for sun exposure and education levels; SNP, single nucleotide polymorphism, VDR, Vitamin D receptor; CYP2R1, Vitamin D 25-hydroxylase (Table 2)

ed previously (3, 12). Since we observed a larger size of the sufficiency group in our results, we estimated this to be due to the difference in where the study was conducted. The previous mentioned studies were conducted with an urban hospital background while our study was set in the medical camps of the rural regions. We found that 50% of the study participants had deficiency in 25(OH)D (< 20 ng/ml). Our findings are lower than the 69.0% and 79.6% prevalence of vitamin D deficiency reported in other regional based studies (15),(16). The differences in the results from the aforementioned studies indicated that although the vitamin D deficiency prevalence is region wide, the affected population proportion is variable.

There are several possibilities that can assist understanding the reasons for insufficient 25(OH)D levels observed in most of the tested sample population. The traditional diet is wheat and grain based and lacks in vitamin D. There is a deficit in clear food fortification policies in Pakistan. Using vitamin D supplementation is not a regular practice in most individuals that we tested. Rural populations in the Punjab province generally have darker skin shades and this can be associated with insufficient vitamin D. Betel nut chewing and consumption of tobacco is a common practice in the study sample that we selected although we did not account for these factors in our study.

The month that we obtained the blood samples was February, which is the winter season of the province of Punjab. The total sunshine hours per month on average in Punjab for the month of February is 271 hours, this number expectedly increases to 304 hours in May. In the month of February, the average day length is approximately 10.6 hours which is shorter when compared to the 13.5 hour days in the summer month of May. The combination of fewer sunshine hours and a shorter day length could mean that individuals in Punjab have lower levels of exposure to sunlight in the month February when compared to the months in the summer season. Low levels of sun exposure in February and winter months prior to it could be the cause of the high number of individuals having insufficiency and deficiency in 25(OH)D as we observed in the present study. This is in line with other studies such as by Goswamin et.al that suggest considerably lower levels of vitamin D in summer than winter months (17). This was corroborated by the Azizi et.al study (18).

On the other hand, there were several individuals in the study with 25(OH)D levels higher than 50 ng/ml. We postulate that since agricultural workers have a variety of exposure to sunshine levels, those who work in the fields with activities such as sowing and reaping are exposed to more sunshine throughout the year when compared to those workers who tend to animals and more involved in harvesting. However detailed information about the exact nature of their agricultural tasks was not obtained from the study participants.

In our study, we noted the parathyroid hormone levels to be significantly different in the 25(OH)D deficiency group versus the sufficiency group. The present study observed 25(OH)D was negatively correlated. The number of individuals with hyperparathyroidism was less than expected despite the large number of individual deficient in vitamin D. This was in contrast to previous studies. Secondary hyperparathyroidism has often been used as a clear indicator of deficiency in vitamin D but we did not find this to be in our study. This could be due to the fact that there were individuals that had low phosphate levels. However, these findings are difficult to corroborate since the study participants were not in the fasting state where more accurate values of phosphate levels can be obtained.(19)

We estimate that the observed low serum phosphate levels could explain the lower PTH levels and there were fewer individuals with secondary hyperparathyroidism. Serum phosphate levels are directly linked to PTH secretion, independent of vitamin D and calcium levels.(20)

Evidence linking vitamin D supplementation as being beneficial has been examined by previous studies such as done by Pelicane et al. (21) When the sufficiency group was compared to the deficiency group (38% vs 21%) in terms of oral supplementation of vitamin D was in contrast to the study by Thomas et al (22) found that using multivitamins did not provide any protection from hypovitaminosis D in hospital admitted patients. However, that study involved acutely ill patients unlike our asymptomatic individuals.

For optimum bone health, the Institute of Medicine (IOM) recommends the daily consumption of vitamin D to be 600 IU/day for adults between 19-70 years of age. This intake is arguably recommended to

ramped up to 1500-2000 IU/day to maintain levels of vitamin D >30 ng/ml. In countries with less sunshine and with an increase in the individual's age, the recommended intake is set at a minimum of 800-1000 IU/day (2). Keeping in view that exposure to sunlight and dietary consumption both do not meet the vitamin D requirements, the need for supplementation is established worldwide but there are no clear regional guidelines currently available about this topic.

The generalizability of our findings is enhanced by the fact that our study involved recruiting participants from three different rural settings. We were also able to obtain a clearer picture on the potential influences on the status of vitamin D such as genetic influences.

Conclusions

In the present study we observed a significant proportion of asymptomatic individuals from the rural population that had low levels of 25(OH)D. Vitamin D deficiency has multifactorial causes and this warrants further investigation for addressing this burgeoning public health issue.

Acknowledgements

The authors wish to express their gratitude to Dr. Fatima, Dr. Zainab, Mr. Tahammal, Ms. Saima, Ms. Rahila and Mr. Zaghum for their invaluable help towards this project.

The study obtained an independent educational grant from the Syed Mohammad Sajjad trust fund which covered the costs of the laboratory tests, equipment usage and other logistics that the study required. The financial grant was approved by the Punjab Care Hospital Research Council (IAC-0497). The funders did not play any role in the design of the study, execution, data collection or analysis and in the manuscript preparation or editing.

All authors of this study have no conflict of interest to declare.

References

1. Lips P. Vitamin D physiology. Progress in biophysics and molecular biology. 2006;92(1):4-8.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism. 2011;96(7):1911-30.
3. Mansoor S, Habib A, Ghani F, Fatmi Z, Badruddin S, Mansoor S, et al. Prevalence and significance of vitamin D deficiency and insufficiency among apparently healthy adults. Clinical biochemistry. 2010;43(18):1431-5.
4. SHERMAN SS, HOLLIS BW, TOBIN JD. Vitamin D Status and Related Parameters in a Healthy Population: The Effects of Age, Sex, and Season*. The Journal of Clinical Endocrinology & Metabolism. 1990;71(2):405-13.
5. Simonelli C. The role of vitamin D deficiency in osteoporosis and fractures. Minnesota medicine. 2005;88(11):34-6.
6. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. The American journal of clinical nutrition. 2008;87(4):1080S-6S.
7. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. The American journal of clinical nutrition. 2004;79(3):362-71.
8. Jurutka PW, Whitfield GK, Hsieh J-C, Thompson PD, Haussler CA, Haussler MR. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. Reviews in Endocrine and Metabolic Disorders. 2001;2(2):203-16.
9. Iqbal R, Khan AH. Possible causes of vitamin D deficiency (VDD) in Pakistani population residing in Pakistan. J Pak Med Assoc. 2010;60(1):1-2.
10. 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. The American journal of clinical nutrition. 2005;81(5):1060-4.
11. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PV, Sarma KV, et al. High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. The American journal of clinical nutrition. 2007;85(4):1062-7.
12. Zuberi LM, Habib A, Haque N, Jabbar A. Vitamin D deficiency in ambulatory patients. Journal of the Pakistan Medical Association. 2008;58(9):482.
13. Badruddin SH, Baig-Ansari N. Overview of Vitamin D and its role in Tuberculosis Prevention and Treatment. Infect Dis J Pakistan. 2008;17:135-8.
14. Thacher TD, Fischer PR, Singh RJ, Roizen J, Levine MA. CYP2R1 mutations impair generation of 25-hydroxyvitamin D and cause an atypical form of vitamin D deficiency. The Journal of Clinical Endocrinology & Metabolism. 2015;100(7):E1005-E13.
15. Harinarayan C, Ramalakshmi T, Venkataprasad U. High prevalence of low dietary calcium and low vitamin D status in healthy south Indians. Asia Pacific journal of clinical nu-

trition. 2004;13(4):359-64.
16. Hashemipour S, Larijani B, Adibi H, Sedaghat M, Pajouhi M, Bastan-Hagh MH, et al. The status of biochemical parameters in varying degrees of vitamin D deficiency. Journal of bone and mineral metabolism. 2006;24(3):213-8.
17. Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. The American journal of clinical nutrition. 2000;72(2):472-5.
18. Azizi E, Pavlotsky F, Kudish A, Flint P, Solomon A, Lerman Y, et al. Serum Levels of 25-Hydroxy-Vitamin D3 Among Sun-protected Outdoor Workers in Israel. Photochemistry and photobiology. 2012;88(6):1507-12.
19. Jubiz W, Canterbury JM, Reiss E, Tyler FH. Circadian rhythm in serum parathyroid hormone concentration in human subjects: correlation with serum calcium, phosphate, albumin, and growth hormone levels. The Journal of clinical investigation. 1972;51(8):2040-6.
20. Lederer E. Regulation of serum phosphate. J Physiol. 2014;592(18):3985-95.
21. Pellicane AJ, Wysocki NM, Schnitzer TJ. Prevalence of 25-hydroxyvitamin D deficiency in the outpatient rehabilitation population. American Journal of Physical Medicine & Rehabilitation. 2010;89(11):899-904.
22. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, et al. Hypovitaminosis D in medical inpatients. New England Journal of Medicine. 1998;338(12):777-83.

Correspondence:
Dr. Nadia Hussain
Department of Pharmaceutical Sciences
Faculty of Pharmacy
Al Ain University of Science and Technology
P.O.Box: 64141, Al Ain, Abu Dhabi
United Arab Emirates
Phone: 0097137024875
Email: nadia.hussain@aau.ac.ae