Journal of Steroid Biochemistry & Molecular Biology 175 (2018) 49–54

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Vitamin D status among the juvenile population: A retrospective study

Afrozul Haq[a,*], Jitka Svobodová[b], Nighat Y. Sofi[c,d], Andrea Jindrová[b], Bohumil Kába[b], Jaishen Rajah[e], Fatme Al Anouti[f], Laila Abdel-Wareth[g], Sunil J. Wimalawansa[h], Mohammed S. Razzaque[i]

[a] Research and Development, Gulf Diagnostic Center Hospital, Abu Dhabi, United Arab Emirates
[b] Department of Statistics, Faculty of Economics and Management, Czech University of Life Sciences, Prague, Kamýcká 129, 165 21 Praha 6—Suchdol, Czechia
[c] All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India
[d] Banasthali University, Rajasthan, India
[e] Institute of Pediatrics, Sheikh Khalifa Medical City, United Arab Emirates
[f] Zayed University, Abu Dhabi, United Arab Emirates
[g] Department of Pathology and Laboratory Medicine, Cleveland Clinic, Abu Dhabi, United Arab Emirates
[h] Endocrinology, Metabolism & Nutrition, Cardio-Metabolic Institute, NJ, USA
[i] Department of Oral Health Policy & Epidemiology, Harvard School of Dental Medicine, Boston, MA, USA

ARTICLE INFO

Article history:
Received 1 November 2016
Received in revised form 8 January 2017
Accepted 10 January 2017
Available online 17 January 2017

Keywords:
Vitamin D deficiency
Juveniles
25(OH)D
Age
Gender
Nationality
United Arab Emirates

ABSTRACT

Vitamin D deficiency is a clinical problem and recently we have shown that 82.5% of our entire study cohort had inadequate serum 25(OH)D levels. In this study, we analysed serum 25(OH)D levels of juvenile patients admitted to the Burjeel Hospital of VPS Health care in Abu Dhabi, United Arab Emirates (UAE) from October 2012 to September 2014. Out of a total of 7883 juvenile patients considered in this study, almost 58.1% of females and 43.3% of males in the age group of 1–18 years were found to have low serum 25(OH)D levels (<50 nmol/L). According to the coefficient of variation, females had significantly higher variability among juveniles (63.8%) than males (49.9%). Among the juveniles group of patients, age appears to be an important determining factor for defining vitamin D deficiency.The risk of deficiency (<30 nmol/L) was found to be present in 31.4% of patients in the age group of 10–12 years, followed by 50.4% of patients in the age group of 13–15 years and 52.9% of patients in the age group of 16–18 years. The analysed age groups of females were found to have lower levels of 25(OH)D than males. It is important and perhaps alarming to note that such high rate of vitamin D deficiency is present in the juvenile age.

1. Introduction

Recently, we have reported that 82% of 60,979 patients part of our comprehensive study have vitamin D deficiency or insufficiency, of which 26% of females and 18% of males have serum 25 (OH)D levels less than 30 nmol/L [1]. Vitamin D deficiency can cause rickets in children or osteomalacia in adults [2–5]. According to assessments, individuals with serum 25(OH)D levels between 30 and 50 nmol/L are at risk of developing bone diseases [6] including osteopenia, osteoporosis with increased rate of fractures in adults. Bone diseases related to vitamin D deficiency, particularly in adolescents with eating disorders,can increase the risk of bone disorders [7]. Studies have also claimed that vitamin D deficiency is associated with increasing risk of cancers, autoimmune diseases, hypertension, and infectious diseases [3]. Nurses' Health Study II provided the evidence on association between higher adolescent serum 25(OH)D level with a lower risk of benign breast disease [8,9]. In children, inadequate 25(OH)D levels can affect the bone mineralization process and accelerate bone dysfunction, earlier than the expected age. Adequate vitamin D status is essential for maintaining musculoskeletal health and beyond; such beneficial effects of vitamin D are partly exerted by intestinal calcium and phosphorus absorption and by maintaining mineral ion homeostasis [4,10–18].

Despite many studies have analysed the status of vitamin D in adults and elderly, sufficient data on the status of serum 25(OH)D

* Corresponding author.
E-mail address: haq2000@gmail.com (A. Haq).

http://dx.doi.org/10.1016/j.jsbmb.2017.01.005
0960-0760/

levels among juveniles of different age groups is not available from the gulf region. Therefore,the present study was conducted to examine the status of vitamin D among the juvenile population of the United Arab Emirates and other countries.

2. Subjects

This study was conducted to determine serum 25(OH)D status of juvenile patients admitted to the Burjeel Hospital in Abu Dhabi, UAE from October 2012 to September 2014. The inclusion criteria considered for the patients were juveniles suffering from diseases without any effect on the serum 25(OH)D levels and patients whose parents/guardians provided consent to participate in the study. Analysed dataset consisted of 60,979 patients and out of these patients, 7883 were juveniles. In this analysis, we included juveniles (patients) in the age group of 1–18 years. The patients were sorted into six equally distributed intervals of age. The serum 25(OH)D levels were estimated in a single laboratory by chemiluminescence technique. The dataset included gender, age and nationality of patients tested for level of serum 25(OH)D. In the analysed group of patients,there were 3586 males (45.5%) and 4297 females (54.5%). 4516 (57.2%) of patients in the data set were UAE nationals and 3367 (42.8%) were patients of other nationalities.

3. Study design and methods

According to the Institute of Medicine (IOM) recommendations, the risk of vitamin D deficiency is defined as serum 25(OH)D level below 30 nmol/L and efficacy started at 25(OH)D levels of at least 50–125 nmol/L [2]. By Anderson-Darling test, it is confirmed that there is not a Gaussian distribution in the data on the significance level of alpha 5%, therefore, the median was used to describe the data and non-parametric tests and Spearman correlation coefficient for quantitative variables [age and level of 25(OH)D]. Spearman correlation coefficient below 0.3 represents a weak dependency, within an interval 0.3–0.8 medium strong dependency and coefficient above 0.8 a strong dependency between variables [19]. Statistical tests that do not assume that the data follow a Gaussian distribution are referred to as non-parametric tests [20]. Non-parametric tests are independent of the population distribution [19]. Qualitative variables (gender, nationality and interval of vitamin D levels) were calculated by coefficients Eta. Coefficient Eta below 0.09 represents no relationship between variables, within an interval 0.10–0.29 weak to medium strong dependency, 0.30-0.49 medium to considerable dependency, 0.50–0.69 considerable to strong dependency, 0.70–0.89 very strong dependency and coefficient above 0.90 is considered to beal most a perfect dependency [1,21]. Statistical softwares SPSS, IBM Statistics 23 and SAS 9.4 have been used for the analysis of data.

3.1. Measurement of total 25(OH)D

The measurement for circulating 25(OH)D is the best diagnostic test for determining a person's vitamin D status [22]. Vitamin D estimation was done by chemiluminescence immunoassay (CLIA), which is a quantitative immunoassay method used for the determination of total 25(OH)D in serum on a fully automated analyser. Currently, the ideal methods for measuring 25(OH)D3 are based on high-performance liquid chromatography (HPLC) or liquid chromatography with tandem mass spectrometry detection (LC–MS/MS). This method simultaneously measures 25(OH)D2 and 25(OH)D3 HPLC and mass spectrometry are capable of differentiating between these two forms, providing distinct results for each of the fractions [23]. In practice, however, automated immunoassays are the methods most commonly used by clinical laboratories [24].

4. Data analysis

Median for 25(OH)D in patients was 47.9 nmol/L. The minimum value of vitamin D in this group of patients was 7.5 nmol/L and maximum 175 nmol/L. 24.6% of patients in the data set had a risk of deficiency of vitamin D (<30 nmol/L) and 26.8% of patients had 30–50 nmol/L. 45.8% of patients had serum 25(OH)D levels within the range of 50–125 nmol/L and the rest of 2.9% had serum 25(OH)D levels greater than 125 nmol/L.

Age and result value of vitamin D were tested by Kruskal-Wallis non-parametric test. By p-value (Sig. = < 0.001) was confirmed at a significance level 0.05 for the alternative hypothesis. That means with probability 95% there is a statistically significant difference between age groups in vitamin D, so age can affect the result value of vitamin D. The age with the result value of vitamin D are medium strong correlated with Spearman correlation coefficient −0.615 at a significance level 0.001.

Gender and result value of 25(OH)D were tested by two-tailed Mann-Whitney test. By p-value (Sig. = < 0.001) the alternative hypothesis is confirmed, that means there is a statistically significant difference between gender and vitamin D at a significance level 0.05, so with probability 95% gender affects the result value of vitamin D. According to coefficient Eta (0.196) there was a weak to medium strong dependency between gender and 25(OH)D.

Nationality and result value of 25(OH)D were tested by Mann-Whitney non-parametric test. By p-value (Sig. = < 0.001) the alternative hypothesis is confirmed, that means there was a statistically significant difference between nationalities and vitamin D at a significance level 0.05, so with probability 95% nationality affects the result value of vitamin D. Coefficient Eta (0.157) showed there is a weak to medium strong dependency between nationality and vitamin D.

5. Results

5.1. Levels of serum 25(OH)D analysed for gender

According to bar chart (Fig. 1), it is obvious that male patients have higher median of 25(OH)D than females in all analysed age groups and this difference between gender is increasing with age from 4 years till 15 years. According to the chart both, males and females, had levels of vitamin D decreasing with increasing age, the blue dotted line depicts the median of 25(OH)D (47.9 nmol/L).

Risk of deficiency (<30 nmol/L) was observed more for females (33.1% of females) than males (14.4% of males), as well the sign scheme revealed significantly more female patients with a level of serum 25(OH)D less than 30 nmol/L (Table 1). Serum 25(OH)D level above 30 nmol/L is seen more frequently in males. The presence of serum 25(OH)D levels (<50 nmol/L) was observed in 58.1% females and 43.3% males. Significantly a higher number of male patients had serum 25(OH)D levels in the range of 50–125 nmol/L.

5.2. Levels of serum 25(OH)D analysed for nationality

UAE nationals had a median serum 25(OH)D level of 43.92 nmol/L and patients from other nationalities had 53.64 nmol/L. A high coefficient of variation was found in both groups of patients. There was a higher variation of vitamin D between juvenile patients in group of UAE nationals(58.9%) compared to other nationalities which was 54.8%.

According to Table 2, the risk of deficiency of 25(OH)D (<30 nmol/L) was found more in UAE nationals (29.5% of UAE nationals) than other countries (18% of other nationalities). A total of 27.5% of UAE nationals and 25.7% patients of other nationalities

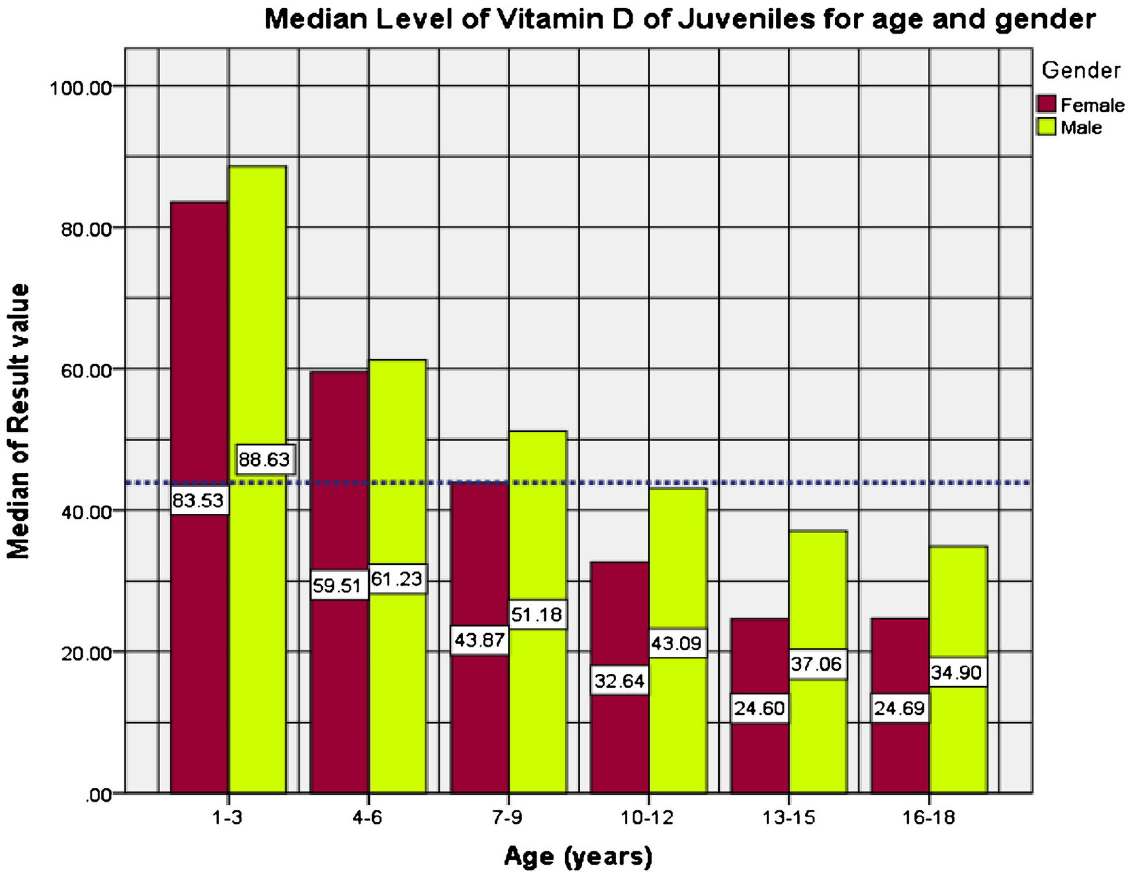

Fig. 1. Median level of 25(OH)D in Juveniles vis-à-vis different age and gender.

were found to be suffering from inadequate levels of vitamin D within the interval of 30–50 nmol/L. The adequate levels (50–125 nmol/L) were found more in the group of other nationals (51.9%) as compared to UAE nationals (41.2%). Potentially for harmful levels of vitamin D (>125 nmol/L) there were 4.4%patients of other nationalities and 1.8% UAE nationals.The sign scheme shows that in UAE nationals suffer significantly more than expected with deficiency and inadequate levels of vitamin D with serum 25(OH)D level less than 50 nmol/L while higher number of patients from other nationalities had serum 25(OH)D levels higher than 50 nmol/L.

5.3. Levels of serum 25(OH)D analysed for age

Juvenile patients were sorted into 6 age groups, the highest median of vitamin D was found in age group of patients less than 3 years old (80.4 nmol/L) and the median was decreasing with increasing age, which was already in the interval of inadequate level (30–50 nmol/L) among patients older than 7–9 years (median 47.4 nmol/L) and risk of deficiency among older than 13–15 years (median 29.3 nmol/L). While higher percentage of patients in the age group of 16–18 years, had low serum 25(OH)D levels and were found to have a higher risk of deficiency (median 28.3 nmol/L). The highest coefficient of variation was found in the age group 16–18

Table 1
Vitamin D cross-tabulation with sign scheme in juveniles vis-à-vis gender.

		Level of 25(OH)D nmol/L				
		Risk of Deficiency (<30)	Inadequate levels(30-50)	Adequate levels (50-125)	Potentially harmful (>125)	Total
Gender	Female	1421	1073	1691	112	4297
	Male	517	1036	1916	117	3586
	Total	1938	2109	3607	229	7883
% within Gender	Female	33.1	25.0	39.4	2.6	100
	Male	14.4	28.9	53.4	3.3	100
	Total	24.6	26.8	45.8	2.9	100
Sign Scheme	Female	+++	---	---	0	
	Male	---	+++	+++	0	

Table 2
Vitamin D cross-tabulationwith Sign Schemein juveniles vis-à-vis nationality.

		Level of 25(OH)D nmol/L				
		Risk of deficiency (<30)	Inadequate levels (30-50)	Adequate levels (50-125)	Potentially harmful (> 125)	Total
Count	UAE	1333	1242	1861	80	4516
	Other	605	867	1746	149	3367
	Total	1938	2109	3607	229	7883
% within nationality	UAE	29.5	27.5	41.2	1.8	100
	Other	18.0	25.7	51.9	4.4	100
	Total	24.6	26.8	45.8	2.9	100
Sign scheme	UAE	+++	o	---	---	
	Other	---	o	+++	+++	

years (89.5%), 13–15 years (78.0%) and 10–12 years (53.9%), a higher variation of level of vitamin D among patients in comparison with other age groups was observed.

Table 3 shows the median levels of 25(OH)D of all 7883 juvenile patients with age groups.

As shown in Table 4, 52.9% of juvenile patients in the age group of 16–18 years and 50.4% juvenile patients in the age group of 13–15 years had deficient serum 25(OH)D (<30 nmol/L). Conversely, there were only 2.2% of patients in the age group 1–3 years with risk of deficiency. The frequency of patients with the risk of deficiency (<30 nmol/L) was increasing with increasing age. Inadequate levels of vitamin D within the interval 30–50 nmol/L was found mostly in age groups of 7–9 years (41.7%) and 10–12 years (40.8%).

On the other hand, lowest number of patients under 3 years of age were found to have inadequate level 30–50 nmol/L (6.7%) however, this age group had the highest percentage of patients with 25(OH)D at a level higher than 50 nmol/L (80.4%). The adequate level was also found for patients in the age group of 4–6 and 7–9 years (69.2% and 44.8%). The lowest number of patients with vitamin D at a level above 50 nmol/L were found in the age groups of 13–15 years (20%) and 16–18 years (20.8%).

In the age group of 1–6 years,a significantly higher number of patients than expected were present with a level of serum 25(OH)D more than 50 nmol/L. Also in the age group of 7–9 years a significantly higher number of patients than expected were present with a level of serum 25(OH)D within an interval 30–50 nmol/L. In the age group 10–12 years significantly higher number of patients than expected had serum 25(OH)D levels less than 50 nmol/L. By coefficient of variation, the highest variation of serum 25(OH)D was found among patients in age group of 13–18 years.

6. Discussion

Adolescence is a period of rapid growth which necessitates the requirement of vitamin D for skeletal growth and development. Vitamin D deficiency is highly prevalent, even in countries with abundant sunshine, when skin exposure to UVB sunlight is limited by lifestyle and other associated factors. The study was conducted on a total of 7883 juvenile patients in the age group of 1 to 18 years selected from a study cohort of 60,979 patients and out of these patients. The prevalence of severe vitamin D deficiency in juveniles is inconsistent, but it is much higher countries, especially in the Middle East and Southeast Asia [25]. The presence of low serum 25 (OH)D levels (<50 nmol/L) in 58.1% females and 43.3% males in our study is similar to vitamin D deficiency documented by earlier studies [26,27]. Age is the most important factor of all studied factors affecting level of vitamin D. With the increase in age, the number of juvenile patients with sufficient levels of serum 25(OH)D (50–125 nmol/L) decreased from 80% in the age group of 1–3 years to only 20.8% for the age group of 16 to 18 years. Recent study on the different ethnic group of adolescent population revealed similar results [28]. The possible mechanism for the decline in the status of serum 25 (OH)D with age is not well understood. A higher percentage (33.1%) of females than males (14.4%) were found to be suffering from vitamin D deficiency in the present study. Similar results were found in studies conducted earlier [29–31]. Karoll et al. [32] studied seasonal variation of 25(OH)D3 for all genders and latitudes. These authors found that vitamin D deficiency and insufficiency was common (33% < 20 ng/mL; 60% < 30 ng/mL) as was elevated iPTH levels (33% > 65 pg/mL). The percentage of patients deficient in 25(OH)D3 seasonally varied from 21% to 48% and the percentage with elevated iPTH reciprocally varied from 28% to 38%.

Our results from a relatively large cohort suggest that an effective strategy to prevent risk of deficiency and inadequate levels of vitamin D is necessary. Juvenile patients with serum 25(OH)D <30 nmol/L

Table 3
Median levels of 25(OH)D in different age groups.

Age (years)	Gender		
	Female (n = 4297), 25(OH)D nmol/L	Male (n = 3586), 25(OH)D nmol/L	Total (n = 7883), 25(OH)D nmol/L
1–3	83.53	88.63	86.14
4–6	59.51	61.23	60.53
7–9	43.87	51.18	47.39
10–12	32.64	43.09	37.70
13–15	24.60	37.06	29.34
16–18	24.69	34.90	28.30
Total	42.37	54.20	47.95

Table 4
Vitamin D cross-tabulation with sign scheme in juveniles vis-à-vis age groups.

		Level of 25(OH)D nmol/L of juveniles				
	Age groups	Risk of deficiency (<30)	Inadequate levels (30-50)	Adequate levels (50-125)	Potentially harmful (> 125)	Total
Count	1-3 years	32	98	1180	157	1467
	4-6 years	70	363	1053	36	1522
	7-9 years	160	509	548	5	1222
	10-12 years	349	453	302	6	1110
	13-15 years	563	323	223	8	1117
	16-18 years	764	363	301	17	1445
Total		1938	2109	3607	229	7883
% within age	1-3 years	2.2	6.7	80.4	10.7	100
	4-6 years	4.6	23.9	69.2	2.4	100
	7-9 years	13.1	41.7	44.8	0.4	100
	10-12 years	31.4	40.8	27.2	0.5	100
	13-15 years	50.4	28.9	20.0	0.7	100
	16-18 years	52.9	25.1	20.8	1.2	100
Total		24.6	26.8	45.8	2.9	100
Sign-scheme	1-3 years	---	---	+++	+++	
	4-6 years	---	--	+++	0	
	7-9 years	---	+++	0	---	
	10-12 years	+++	+++	---	---	
	13-15 years	+++	0	---	---	
	16-18 years	+++	0	---	---	

were provided with a weekly oral dose of vitamin D supplementation (50,000 IU/week). Both observational and experimental studies have reported benefits of vitamin D supplementation and increased exposure to safe sunlight as a way to increase 25(OH)D synthesis to reduce the risk of musculoskeletal diseases. Since hypovitaminosis of D status usually reflects reduced sunlight exposure [10], the obvious primary replacement should be safe sunlight exposure, when available. Of relevance, safe sunlight exposure can reduce the bone deformities including rickets and osteoporosis. A higher risk for rickets due to lack of sunlight exposure was noted more than a century ago [33]. Finally, as our studied cohort of juvenile patients were admitted patients in the hospital, it is difficult to determine whether inadequate vitamin D status is a cause or a consequence of their diseases. More studies are required to be conducted especially in the Middle East and Southeast Asia regions to strengthen the findings of the present study.

Conflict of interest

The authors have declared no conflict of interests.

Acknowledgement

This work was supported by the Faculty of Economic and Management, Czech University of Life Sciences under IGA Grant No. 20151035.

References

[1] A. Haq, J. Svobodova, S. Imran, C. Stanford, M.S. Razzaque, Vitamin D deficiency: a single centre analysis of patients from 136 countries, J. Steroid Biochem. Mol. Biol. 164 (2016) 209–213, doi:http://dx.doi.org/10.1016/j.jsbmb.2016.02.007.
[2] IOM, Dietary Reference Intakes for Calcium and Vitamin D, The National Academies Press, Washington, DC, 2011.
[3] M.F. Holick, T.C. Chen, Vitamin D deficiency: a worldwide problem with health consequences, Am. J. Clin. Nutr. 87 (2008) 1080S–1086S.
[4] B. Lanske, M.S. Razzaque, Vitamin D and aging: old concepts and new insights, J. Nutr. Biochem. 18 (2007) 771–777.
[5] R.A. Sulimani, A.G. Mohammed, A. Alfadda, S.N. Alshehri, A.M. Al-Othman, N. M. Al-Daghri, D.A. Hanley, A.A. Khan, Vitamin D deficiency and biochemicalvariations among urabn Saudi adolescent girls according to season, Saudi Med. J. 37 (2016) 1002–1008.
[6] S.H. Pearce, T.D. Cheetham, Diagnosis and management of vitamin D deficiency, BMJ 340 (2010) b5664.
[7] D. Modan-Moses, Y. Levy-Shraga, O. Pinhas-Hamiel, B. Kochavi, A. Enoch-Levy, I. Vered, D. Stein, High prevalence of vitamin D deficiency and insufficiency in adolescent inpatients diagnosed with eating disorders, Int. J. Eat. Disord. 48 (2015) 607–614.
[8] X. Su, G.A. Colditz, L.C. Collins, H.J. Baer, L.A. Sampson, W.C. Willett, C.S. Berkey, S.J. Schnitt, J.L. Connolly, B.A. Rosner, R.M. Tamimi, Adolescent intakes of vitamin D and calcium and incidence of proliferative benign breast disease, Breast Cancer Res. Treat. 134 (2012) 783–791.
[9] C.E. Boeke, R.M. Tamimi, C.S. Berkey, G.A. Colditz, E. Giovannucci, S. Malspeis, W.C. Willett, A.L. Frazier, Adolescent dietary vitamin D and sun exposure in relation to benign breast disease, Cancer Causes Control 26 (2015) 1181–1187.
[10] M.S. Razzaque, Sunlight exposure: do health benefits outweigh harm? J. Steroid Biochem. Mol. Biol. 175 (2018) 44–48, doi:http://dx.doi.org/10.1016/j.jsbmb.2016.1009.1004.
[11] R.B. Brown, M.S. Razzaque, Phosphate toxicity: a stealth biochemical stress factor? Med. Mol. Morphol. 49 (2016) 1–4.
[12] M.S. Razzaque, B. Lanske, Hypervitaminosis D and premature aging: lessons learned from Fgf23 and Klotho mutant mice, Trends Mol. Med. 12 (2006) 298–305.
[13] R.B. Brown, A. Haq, C.F. Stanford, M.S. Razzaque, Vitamin D, phosphate, and vasculotoxicity, Can. J. Physiol. Pharmacol. 93 (2015) 1077–1082.
[14] R.B. Brown, F. Al Anouti, M.S. Razzaque, Vitamin D supplements: magic pill or overkill? South East Asia J. Public Health 5 (2015) 1–3.
[15] C. Zhou, F. Lu, K. Cao, D. Xu, D. Goltzman, D. Miao, Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice, Kidney Int. 74 (2008) 170–179.
[16] M.S. Razzaque, Bone-kidney axis in systemic phosphate turnover, Arch. Biochem. Biophys. 561 (2014) 154–158.
[17] M.S. Razzaque, Phosphate toxicity: new insights into an old problem, Clin. Sci. (Lond.) 120 (2011) 91–97.
[18] M.S. Razzaque, The dualistic role of vitamin D in vascular calcifications, Kidney Int. 79 (2011) 708–714.
[19] P. Hoskova, A. Jindrova, M. Pasilova, R. Zeipelt, I. Statistika, Czech Univ. Life Sci. 3 (2012) (ISBN 978-980-213-2341-2344).

[20] A. Kaura, Crash Course Evidence-Based Medicine: Reading and Writing Medical Papers, Mosby Ltd., 2013, pp. 1–172 (ISBN: 9780723438694).
[21] P. Mares, L. Rabusic, P. Soukup, Analýzasociálněvědníchdat (nejen) v SPSS, Masaryk University, Brno, 2015 (ISBN 978-980-210-6362-6364).
[22] S. Abbas, J. Linseisen, J. Chang-Claude, Dietary vitamin D and calcium intake and premenopausal breast cancer risk in a German case–control study, Nutr. Cancer 59 (1) (2007) 54–61.
[23] N.J. Schuch, V.C. Garcia, L.A. Martini, Vitamin D and endocrine diseases, Arq. Bras. Endocrinol. Metab. 53 (2009) 625–633.
[24] L. Abdel-Wareth, A. Haq, A. Turner, S. Khan, A. Salem, F. Mustafa, N. Hussein, F. Pallinalakam, L. Grundy, G. Patras, J. Rajah, Total vitamin D assay comparison of the Roche diagnostics vitamin D total electrochemiluminescence protein binding assay withthe chromsystems HPLC method in a population with both D2 & D3 forms of vitamin D, Nutrients 5 (2013) 971–980.
[25] M.F. Holick, Vitamin D: a millenium perspective, J. Cell Biochem. 88 (2003) 296–307.
[26] G. Marrone, I. Rosso, R. Moretti, F. Valent, C. Romanello, Is vitamin D status known among children living in Northern Italy, Eur. J. Nutr. 51 (2012) 143–149.
[27] J. Oberg, R. Jorde, B. Almas, N. Emaus, Grimnes G. Scand, Vitamin D deficiency and lifestyle risk factors in a Norwegian adolescent population, J. Public Health 42 (2014) 593–602.
[28] G.L. Khor, W.S. Chee, Z.M. Shariff, B.K. Poh, M. Arumugam, J.A. Rahman, H.E. Theobald, High prevalence of vitamin D insufficiency and its association with BMI-for-age among primary school children in Kuala Lumpur, Malaysia, BMC Public Health 11 (2011) 95.
[29] K. Joshi, V. Bhatia, Vitamin D deficiency in a tropical country–treatment and prevention in children, Indian J. Pediatr. 81 (2014) 84–89.
[30] M. González-Gross, J. Valtueña, C. Breidenassel, L.A. Moreno, M. Ferrari, M. Kersting, S. De Henauw, F. Gottrand, E. Azzini, K. Widhalm, A. Kafatos, Y. Manios, P. Stehle, HELENA Study Group, Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study, Br. J. Nutr. 107 (2012) 755–764.
[31] N.M. Al-Daghri, Y. Al-Saleh, N. Aljohani, M. Alokail, O. Al-Attas, A.M. Alnaami, S. Sabico, M. Alsulaimani, M. Al-Harbi, H. Alfawaz, G.P. Chrousos, Vitamin D deficiency and cardiometabolic risks: a juxtaposition of Arab adolescents and adults, PLoS One 10 (7) (2015) e0131315.
[32] M.H. Kroll, C. Bi, C.C. Garber, H.W. Kaufman, D. Liu, A. Caston-Balderrama, K. Zhang, N. Clarke, M. Xie, R.E. Reitz, S.C. Suffin, M.F. Holick, Temporal relationship between Vitamin D status and parathyroid hormone in the United States, PLoS One 10 (3) (2015) e0118108, doi:http://dx.doi.org/10.1371/journal.pone.0118108.
[33] A.F. Hess, The prevention and cure of rickets by sunlight, Am. J. Public Health (N. Y.) 12 (1922) 104–107.