www.jmscr.igmpublication.org
Impact Factor 5.244
Index Copernicus Value: 5.88
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: http://dx.doi.org/10.18535/jmscr/v4i6.57

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

A Comparative Study of Vitamin D and Serum Total Calcium Levels in Two Socioeconomic Groups in Guwahati Metropolitan City

Authors
Dr Dibyaratna Patgiri, Dr Syeda Mohsina Rohman, Dr Mousumi Borgohain Borah
Department of Biochemistry, Gauhati Medical College & Hospital, Guwahati, Assam – 781032 India
Corresponding Author
Dr Dibyaratna Patgiri
Demonstrator, Dept of Biochemistry,
Gauhati Medical College & Hospital, Guwahati, Assam 781032 India
Email: *dibyaratnapatgiri@gmail.com, Mobile Phone – 9854876409, 9859225038*

Abstract
The aim of this cross-sectional study was to assess the Vitamin D and Total Calcium levels among two socio-economic groups in the city of Guwahati. The study included 180 participants divided equally into two groups – Office group and Wage-earner group. Serum Vitamin D was assessed by ELISA using BIORAD microplate reader and Serum Calcium by spectrophotometry using Vitros 5600 Fully autoanalyzer. Unpaired t-test results showed statistically significant decreased levels of both Vitamin D and Total Calcium in individuals in the Office group as compared to those in the Wage-earner group ($p < 0.05$). Females, irrespective of the group, had statistically significant lower levels of both Vitamin D and Total Calcium as compared to males ($p < 0.05$). The study concludes that lifestyle factors related to work are affecting the Vitamin D status in the population and that Vitamin D insufficiency and deficiency is more prevalent among females.
Key words: *Vitamin D, Total Calcium, Sunlight exposure.*

INTRODUCTION

Vitamin D, also known as Calciferol, is not strictly a vitamin, since it can be synthesized in the skin[1]. Under normal circumstances, 80-90% of Vitamin D is synthesized in the skin from 7-dehydrocholesterol present in the Malpighian layer of epidermis on exposure to sunlight[2]. Only when sunlight exposure is inadequate, a dietary source of Vitamin D becomes necessary and it contributes to 10-20% of the total Vitamin D pool[1,2]. 30 minutes of exposure of skin over arms and face to sunlight between 10am to 2pm in regions lying between 42ºN and 42ºS is adequate to avoid Vitamin D deficiency[3].

The main biological function of Vitamin D is regulation of Calcium absorption and homeostasis through its actions on kidney, bones, parathyroid and intestine. This in turn sustains bone mineralisation, muscle contraction, nerve conduction and general cellular function in all cells of the body. The active form of Vitamin D, 1,25-dihydroxyvitamin D or Calcitriol mediates

by way of nuclear receptors to regulate gene expression and hence play a role in regulating cell proliferation and differentiation[1]. These actions of Vitamin D are termed as 'genomic effects' or 'Calciotropic effects'[4]. Calcitriol also has some biologic effects which occur too rapidly for transcriptional mechanisms to be implicated. These 'nongenomic actions' or 'pleiotropic effects', including a rapid increase in intracellular calcium, activation of phospholipase C, and opening of calcium channels, are observed in several cell types within minutes of exposure to Calcitriol[5].

Synthesis of Vitamin D starts by non enzymic photoreaction on exposure of skin to sunlight (UVB rays). This leads to conversion of 7-dehydrocholesterol to previtamin D, which then undergoes two sequential hydroxylation reactions in the liver and kidneys to yield first 25-hydroxyvitamin D, the chief circulating form and then 1,25-dihydroxyvitamin D, the biologically active form[1,5].

Vitamin D deficiency as well as excess is both known to cause a variety of diseases. Deficiency causes rickets in children due to decreased mineralisation of bones and osteomalacia in adults characterised by demineralisation of bone[1]. Moreover a variety of diseases viz. obesity, cancer of colon, breast, prostate, ovary and oesophagus, diabetes, and hypertension have been linked to inadequate Vitamin D and Calcium metabolism[6,7]. On the other hand, elevated plasma levels of Vitamin D results in elevation of serum calcium levels leading to contraction of blood vessels, high blood pressure and calcinosis[1].

MATERIALS AND METHODS

Guwahati city is located at 26.18ºN and 91.73ºE latitude and longitude. Samples were collected all through the year. The average daily duration of sunshine is 10 hours. The study consisted of 180 participants. The participants were divided into two equal groups. The Office executives and staff constituted Group I called the Office Group and the daily wage earners constituted Group II called the Wage Earner Group.

Exclusion criteria

Certain exclusion criteria were applied during sample collection to avoid bias. Individuals with hepatic, renal, dermatological and endocrine disorders which could affect Vitamin D levels were excluded. Additionally, pregnant and lactating women, children, alcoholics, cigarette smokers, those with any acute or terminal illness or those taking any medications which could affect levels of Vitamin D were excluded from the study.

Questionnaire

The details regarding the diet consumed and the average duration of sunlight exposure were collected with the help of a self-reporting questionnaire. The questionnaire incorporated questions regarding the type and amount of food consumed in the last 24 hours, time spent outdoors during work or commuting to and from office, duration of sunlight exposure between 10 am and 2 pm and the type of occupation.

Sample Collection

5 mL blood sample was drawn from the participants under aseptic conditions from the median cubital vein. It was collected in properly labelled vacutainers and then centrifuged at 3000 rpm for 15 minutes. The serum thus obtained was subjected to biochemical analysis within 8 hours of collection of blood.

Estimation Of Vitamin D[8]

Vitamin D estimation was done by ELISA using 25-OH Vitamin D ELISA kit obtained from Euroimmun Medizinische Labordiagnostika, Germany. In the analysis step, the calibrators and patient samples are diluted with biotin-labelled 25-OH vitamin D and added to microplate wells coated with monoclonal anti-25-OH vitamin D antibodies. During the incubation an unknown amount of 25-OH vitamin D in the patient sample and a known amount of biotin-labelled 25-OH vitamin D compete for the antibody binding sites in the microplate wells plate. Unbound 25-OH vitamin D is removed by washing. For the

detection of bound biotin-labelled 25-OH vitamin D, a second incubation is performed using peroxidise-labelled streptavidin. In a third incubation using the peroxidise substrate tetramethylbenzidine (TMB) the bound peroxidise promotes a colour reaction. The colour intensity is inversely proportional to the 25-OH vitamin D concentration in the sample. Results for the samples are then calculated directly using a standard curve generated using calibrators supplied with the kit.

Estimation Of Total Serum Calcium[9]

Estimation of Total serum calcium was done by spectrophotometry using the Vitros 5600 Integrated system autoanalyzer. The VITROS Ca Slide method is performed using the VITROS Ca Slides and the VITROS Chemistry Products Calibrator Kit 1 on the VITROS 5600 Integrated System. The VITROS Ca Slide is a multilayered, analytical element coated on a polyester support.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The bound calcium is dissociated from binding proteins, allowing the calcium to penetrate through the spreading layer into the underlying reagent layer. There, the calcium forms a complex with Arsenazo III dye, causing a shift in the absorption maximum. After incubation, the reflection density of the coloured complex is measured spectrophotometrically. The amount of coloured complex formed is proportional to the calcium concentration in the sample.

Table. 1: Test characteristics of total calcium reagent

Test Type	System	Approx. Incubation Time	Temperature	Wavelength	Reaction Sample Volume
Colorimetric	Vitros 5600	5 Minutes	37ºC(98.6 ºF)	682 nm	10 µL
Ca^{+2} + Arsenazo III		(pH 5.6) ⟶			Coloured Complex

Ethics

The study was approved by the Institutional Ethics Committee, Gauhati Medical College & Hospital and informed written or verbal consent was obtained from all the participants in the study.

Statistics

All data was expressed as Mean ± SD. Unpaired t-test was performed to evaluate whether there was any statistically significant difference in the mean Vitamin D and Calcium levels among the two different groups. $P<0.05$ was considered to indicate statistical significance. Statistical analysis was performed using IBM SPSS Statistics Program Version 20.0.0.

RESULTS AND DISCUSSION

Fig. 1 shows the calibration data for the Vit D kit performed on the ELISA plate reader (Model No. 680) of BIORAD.

Fig. 2 shows the statistical comparison between the mean Vit D levels between the Office group and the Wage-earner group. The individuals in the Office group has statistically significant lower level of Vit D as compared to those in the Wage-earner group.

Fig. 3 shows the statistical comparison between the mean Serum Calcium levels between the Office group and the Wage-earner group. The individuals in the Office group had statistically significant lower level of Serum Calcium as compared to those in the Wage-earner group.

Fig. 4 shows the statistical comparison between the Vit D levels between males and females in the study irrespective of group. Females had a statistically significant lower level of Vit D as compared to males.

Fig. 5 shows the statistical comparison between the Serum Calcium levels between males and females in the study irrespective of group.

Females had a statistically significant lower level of Serum Calcium as compared to males.

Our study shows a statistically significant lower level of Vitamin D in the office going population as compared to the wage-earner population. This observation corroborates findings of other researches that have found a significant rural–urban variation in Vitamin D status[3, 10]. It has been widely publicised that there is rampant Vitamin D deficiency in the general population as a whole but there has been no study as yet on the comparative Vitamin D status among different sections of the population. Various studies have found that sun exposure is a significant predictor of Vitamin D status[10, 11]. We have found similar observations on comparing the two groups studied.

We have also found that women, irrespective of profession, had significantly lower levels of Vitamin D as compared to men. This has been attributed by different authors [11, 12, 13] to different practices regarding clothing, sunscreen use and type of work of the female population. Our findings are in keeping with those from other studies[10, 12, 13].

The calcium absorption from the gastrointestinal tract is a function of an individual's Vitamin D status[14]. With respect to the observations of Vitamin D status in the two groups studied, we have found that it could be reflected in the statistically significant low total serum calcium levels in the office group as compared to the wage-earner group. This relation between Vitamin d and body Calcium has been reported by other researchers as well[15, 16, 17].

A limitation of the present study was that we were not been able to include a larger population which is representative of all socio-economic sections of the society. This involves significant amount of funding which was lacking in the present study. A larger sample would have also made it possible to find out the seasonal variation in the Vitamin D status of the population. We hope our study would be of some benefit to bring to knowledge the Vitamin D status in this part of the country.

Fig. 1: Calibration of Vitamin D Reagent

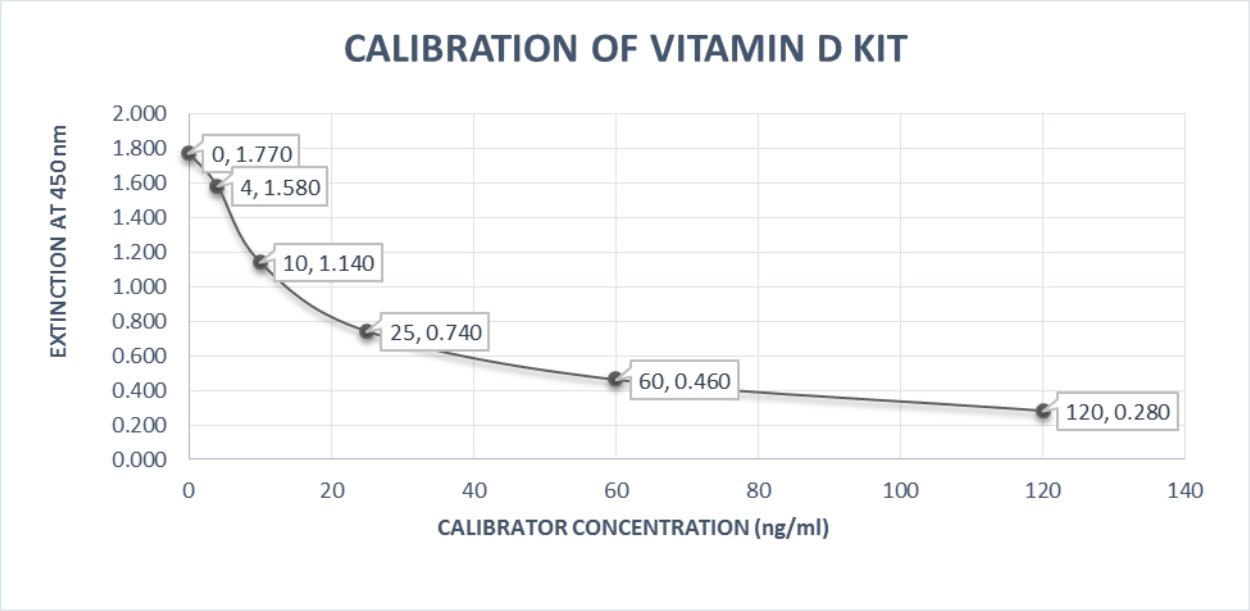


Fig. 2: Mean Vitamin D levels in the study population

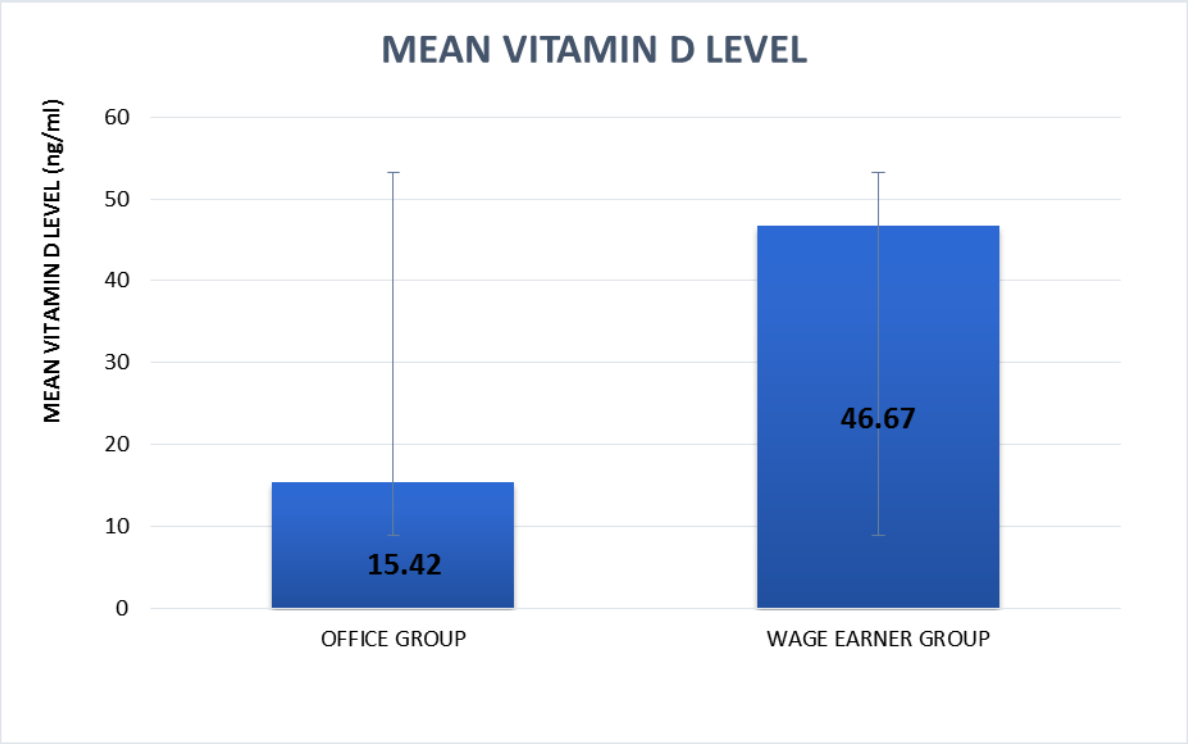


Fig. 3: Mean Total Calcium levels in the study population

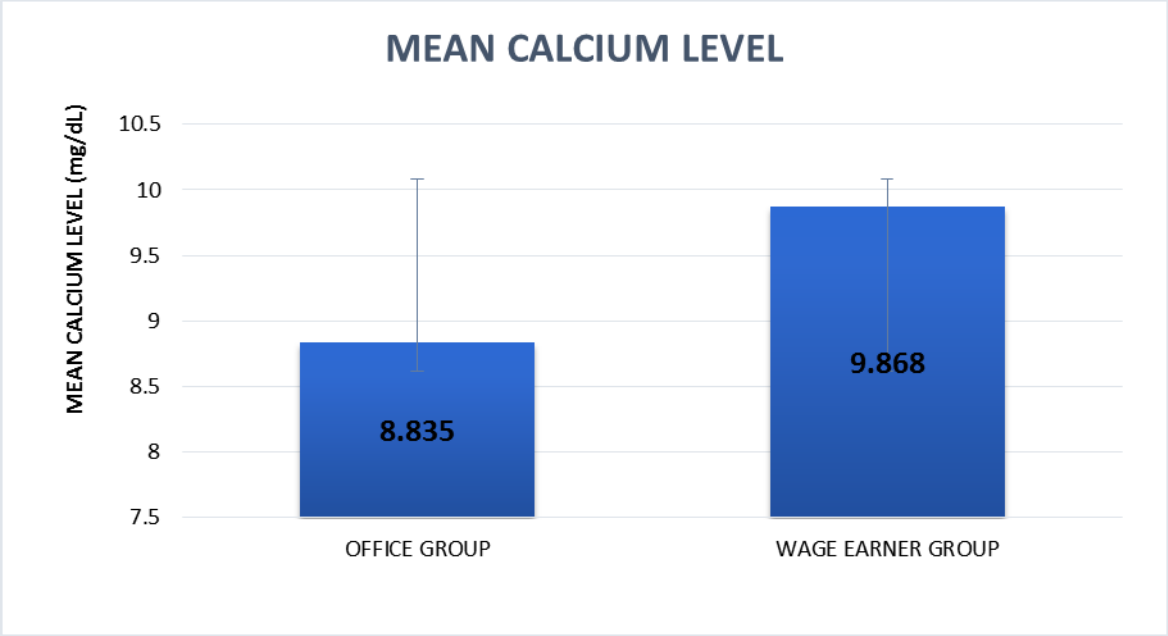


Fig. 4: Mean Vitamin D levels among Males and Females

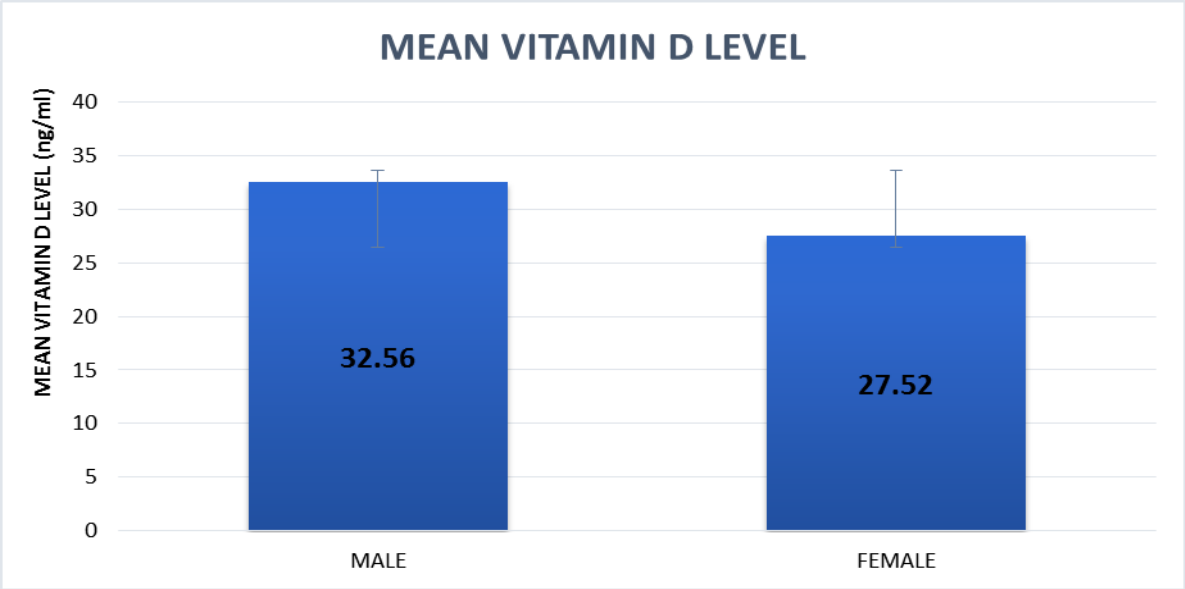
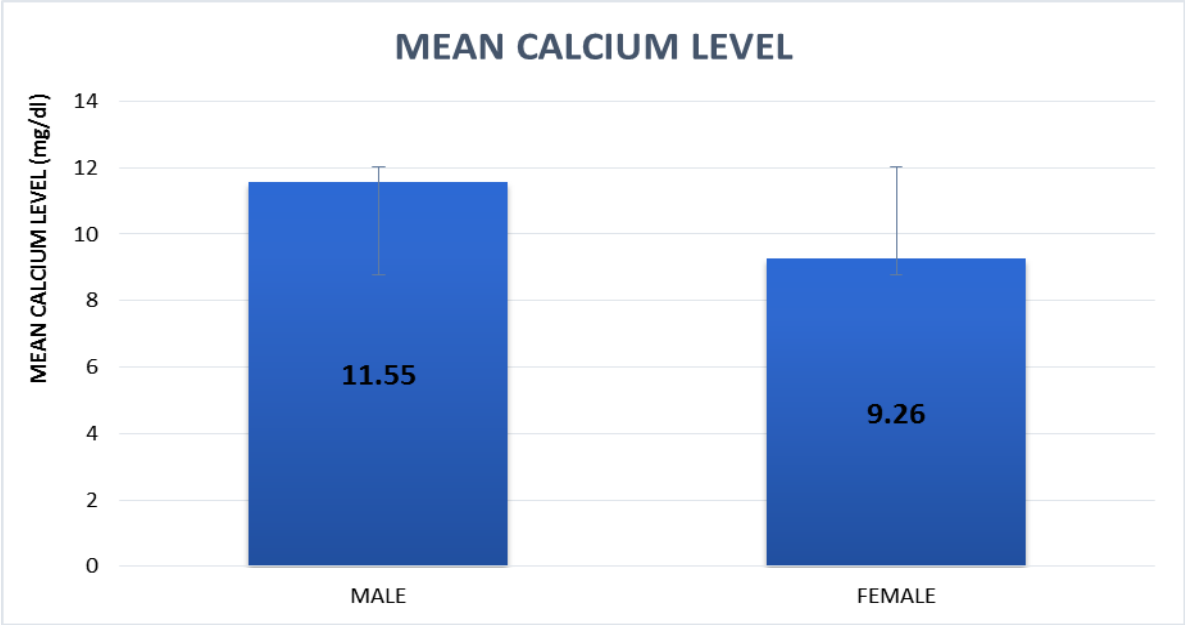


Fig. 5: Mean Total Calcium levels among Males and Females



CONCLUSION

Our results suggest that the influence of life-style factors is affecting the Vitamin D status of the population. Vitamin D insufficiency and deficiency are more prevalent among females. It probably reflects greater avoidance of sunlight among women due to the use of sunscreens or due to their work indoors. Because systemic vitamin D status is determined primarily by the amount of vitamin D produced in skin due to sunlight exposure and not only on dietary sources alone, people who habitually participated in outdoor activities due to work had higher vitamin D levels than those who did not participate in such activities. Vitamin D deficiency and its health consequences is a worldwide health problem. This observation becomes important in context to the Governmental agencies which have not yet taken up any programme for Vitamin D supplementation or fortification. Though it is true that excessive exposure to sunlight and sun burn increases risk of skin cancer and wrinkling, there is little evidence that sensible sun exposure throughout life which would promote adequate vitamin D stores would significantly increase the risk of the either.

REFERENCES

1. Bender DA. Ch 44, Micronutrients: Vitamins and Minerals. In: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA, editors. Harper's Illustrated Biochemistry. 29th ed.,New York; McGraw-Hill Medical; 2009, p. 525-42.
2. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA et al. Global vitamin D status and determinants of hypovitaminosis D. OsteoporosInt, 2009 Nov;20(11):1807-20.
3. Londhey V. Vitamin D Deficiency: Indian Scenario. JAPI, 2011 Nov;59:695-96.
4. Rathi N, Rathi A. Vitamin D and Child Health in the 21st Century. Indian Pediatrics, 2011 Aug;48:619-25.
5. Bringhurst FR, Demay MB, Kronenberg HM. Ch 28, Hormones and Disorders of Mineral Metabolism. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, editors. Williams Textbook of Endocrinology. 12th ed.; Philadelphia; Elsevier Saunders; 2011, p. 1237-1304.
6. Martini LA, Verly E, Marchioni DML, Fisberg RM. Prevalence and correlates of calcium and vitamin D status adequacy in adolescents, adults, and elderly from the Health Survey - Sao Paulo. Nutrition, 2013; 29:845–850.
7. Holick MF. Vitamin D: Important for Prevention of Osteoporosis, Cardiovascular Heart Disease, Type 1 Diabetes, Autoimmune Diseases, and Some Cancers. Southern Medical Journal, 2005 Oct;98(10):1024-27.
8. 25-OH Vitamin D ELISATest instruction. EQ_6411_A_US_D01.doc. [products insert]. Euroimmun US. Version: 07/08/2013 10:16:41.
9. VITROS Chemistry Products Ca Slides. REF 145 0261. [products insert]. Ortho-Clinical Diagnostics, Inc. document: Pub. No. MP2-10 Version 7.0.
10. Vierucci F, Pistoia MD, Fanos M, Erba P, Saggese G. Prevalence of hypovitaminosis D and predictors of vitamin D status in Italian healthy adolescents. Italian Journal of Pediatrics; 2014, 40:54.
11. Elsammak MY, Al-Wossaibi AA, Al-Howeish A, Alsaeed J. High prevalence of vitamin D deficiency in the sunny Eastern region of Saudi Arabia: a hospital-based study. Eastern Mediterranean Health Journal, 2011; 17(4):317-22.
12. Holvik K, Brunvand L, Brustad M, Meyer HE. Vitamin D status in the Norwegian population. In: Solar Radiation and Human Health. EspenBjertness, editor. Oslo: The Norwegian Academy of Science and Letters, 2008.
13. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clinical Endocrinology, 2012; 77:26–35.
14. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesis vitamin D3. Lancet, 1982; 1:74–75.
15. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am CollNutr, 2003; 22:142–46.
16. Heaney RP. Vitamin D depletion and effective calcium absorption. A letter to the editor. J Bone Min Res, 2003; 18:1342
17. Pettifor JM. Vitamin D &/or calcium deficiency rickets in infants and children; a global prespective. Indian J Med Res, 2008; 127: 245-9.