Alterations of Vitamin "D" level in Sera of Iraqi Population

Ahmed M.Issa Sana A. Ibraheem
Kufa university college of Medicine

Abstract:

Vit D is a steroid aid to maintain the normal level of calcium in the circulation. It was measured by the high performance liquid chromatography technique. This technique was of choice in measuring the low levels of vit D in sera. In the current study 60 person (29 males and 31 females) enrolled to assess the level of this vitamin in their bodies. The main level of vit D was 19.21 ng/ml in both sexes and there are many significant differences between males and females in addition to a noticeable change in vit D level due to seasonal variation during summer and winter. The study emphasized on the importance of sun rays as a necessary tool to enhance the level of vit D and prevent vit D deficiency in both sexes

الخلاصه:

يعتبر فيتامين د من الاستيرويدات التي تحافظ على مستوى عنصر الكالسيوم في الدورة الدمويه و قد تم قياسه بواسطة تقنية كورموتوكرافيا السائل عاليه الاداء. ان هذه التقنية هي الانسب لقياس التراكيز الواطئه من هذا الفيتامين في الدم.في هذه الدراسة تم فحص ٦٠ شخصا (٢٩ ذكور و ٣١ اناث) لتحديد مستوى فيتامين د في امصالهم. لقد كان معدل فيتامين د مساوي الى ١٩.٢١ نانوغرام لكل لتر في كلا الجنسين كما ظهرت اختلافات واظحة بالمقارنة بين الرجال و النساء بالاضافة للفارق الملحوظ بين الصيف و الشتاء. لقد شددت الدراسة على اهمية ضوء الشمس كاداة ضرورية لزيادة مستوى فيتامين د و منع نقصه المحتمل في كلا الجنسين.

Introduction:

Vitamin D is a steroid (steroids are a 17 carbone atoms cyclopentano perhydrophenanthrene) prohormone, also known as cholecalciferol . Vitamin D also indexed as, Calciferol, calcipotriol, ergocalciferol,and (Vitamin D2) (1). The most important role of vitamin D is the maintaining of normal calcium levels in blood, which it accomplishes by increasing absorption of calcium from food and reducing urinary calcium loss. Both effects keep calcium in the body and therefore spare the calcium that stored in bones. When necessary, Vitamin D mediate the transport of calcium from the bone in to the blood stream, which does not benefit bones. Although the over all effect of vitamin D on the bone is complicated, vitamin D is necessary for healthy bones and teeth (1). The relations between calcium absorption and vitamin D are similar to that of a locked door and key. Vitamin D is the key that unlocks the door and allows calcium to leave the intestine and enter the blood stream. Vitamin D also work in the kidneys to help resort calcium that otherwise would be excreted (2). Vitamin D is maintaining serum calcium and phosphorus levels within the normal range to sustain a wide variety of metabolic and physiological functions, including maintaining bone health and normal neuromuscular function (3). Adequate vitamin D is required to up regulate calcium absorption in times of increased requirement (growth, pregnancy) and when dietary calcium intake is low(2).Vitamin D also appears to have other roles within the body, such as enhancing immune function(4), blood cell formation ,also helps cells "differentiate" a process that may reduce the risk of cancer(5).From animal and human studies, researchers have hypothesized that vitamin D may protect people from multiple sclerosis(1), auto immune arthritis, and juvenile diabetes(5).Vitamin D comes from food, but sun light is essential to

convert it to its active form. Naturally occurring vitamin D is rare in foods. Vitamin D is used to refer to either vitamin D2 or vitamin D3, both of which can be converted to active vitamin D metabolites. Vitamin D2 originates from the yeast and plant sterol ergosterol, whereas vitamin D3 originates from 7-DHC [7- Dehydrocholesterol] in animals. The major structural difference between vitamin D2and vitamin D3 is in the side chain. Unlike vitamin D3, vitamin D2 has a double bond between carbon number 22 and 23 and a methyl group at carbon number 24(fig.2)(6).The major natural sources of vitamin D are fatty fish like mackerel salmon and fish oils, including cod and tuna liver oils.The major dietary sources of vitamin D are foods that fortified with vitamin D. In the United States since 1930s as example, some cereals and breads have been fortified with vitamin D. Other dairy products, including ice cream, cheeses, and yoghurt, are not fortified with vitamin D. The vitamin D content in milk, however, is variable. Also vitamin D is found in egg yolk and butter (6).

Materials and methods: During both summer and winter seasons 60 blood samples (29 males and 31females) were collected. After with drawing 5 ml of venous blood , the blood transferred to plastic anticoagulant free tube, then left for several minutes to clot and centrifuged by the centrifuge for 15 minutes with 1600 xg Then 3 ml of separated serum was isolated.One milliter was used from this serum to determine 25- hydroxy vitamin D concentration. Also 0.01ml from this separated serum was used for calcium determination. The serum stored at deep freeze -20C until the time of analysis, to prevent any unfavorable decomposition. When the time for analysis had arrived the serum was kept at room temperature with suitable time. The HPLC separation works with an isocratic method at 35C and reversible phase column was applied .Chromatograms aredetected by the UV-detector.The retention time of 25-hydroxy vitamin D was 20 minutes for each run.Results are quantified by the delivered serum calibrator andcalculated by the external standard method by integration of the peak area. The acetonitrile solution was used as eluant solution.Due to the affinity of 25-hydroxyvitamin D3 to plastic all steps was performed in glass tubes, preferable pointed-bottom glass tubes (7).

The analytical Conditions for Vitamin D measurement were as follow.

A-Mobile Phase: 100 % Acetonitrile. B-Flow Rate: 1 ml / min. C- Wave Length : 256nm- 262nm. D- Column shimpack: MRC- ODS [Octadecylslyle]. E- Temperature : Room temperture.

The calcium kit was contained many reagents that purchased from biomerx SA marcy –I Etoil / france Total calcium in sera was determined by a colorimetric method. Without deproteinization calcium ion reacts with the methylthymol blue indicator [MTB] in an alkaline medium. Ca + MTB----- Ca-MTB complex The color intensity of theCa-MTB complex, was measured at 612nm, and it was Proportional to the quantity of calcium present in the sample.

Statistical Analysis.

In this search the usual statistical methods were uses in order to analyze and asses the obtained results.

1- Arithmetic mean (x).
2-Standard deviation (SD).
3- Coefficient of variation (C.V %).
4- Student (t-test), significant variation was considered when p value was less than 0.05.

Results and Discussion:

The study primerly was intended to adapt a method for measuring vitamin D levels in serum samples in order to make a reliable evaluation of this vitamin in Iraqi Population (Najaf

governorate). The HPLC method was the method of choice in this field .The reverse phase HPLC chromatographic method has a very high sensitivity and need only for a very small amount of sample without any complicated pretreatment for the used serum which may be a major source of errors.

The HPLC technique become more widely used during the past 10 years, especially for measuring the different species of vitamin D and vitamin D metabolites in human (8).Many points were strictly followed during the measurement of vitamin D for example , any hemolytic samples were excludedfrom the measured groups, all samples were collected during the morning at about 8:00-10.00 o clock and all patients andvolunteers were, (as they mentioned), did not taken any medicament that may influence vitamin D levels which may alter the final results. The mean of vitamin D levels in both sexes and in different seasons was 19.21 ng/ml the other related statistical parameters and serum calcium level are shown in table 2.

Table 2: Vitamin D Level in Sera of Healthy Males and Females.

Group Type	Vitamin D ng/ml				Calcium mg/dl		
	No.	X	SD	CV%	X	SD	CV%
Male	29	24.56	5.52	22.47	10.87	2.22	20.45
Female	31	14.37	1.86	12.93	10.72	2.22	20.70
Total	60	19.21	6.51	33.91	10.79	2.19	20.32

This value in fact can be relatively considered as sub-normal value. Holick.2000 (9) reported that the normal range of serum 25-hydroxyvitamin D level in human is 25-56 ng/ml. In addition he mentioned that the optimal 25-hydroxyvitamin D value is 45-50 ng/ml. However, this result was comparable with reference value for serum 25-hydroxyvitamin D concentration that obtained from Australia and New Zealand where the 25-hydroxyvitaminD level ranging 10-20 ng/ml (10). In U.S.A. it was reported that 70%of the United State population has 25-hydroxyvitamin D level below 35 ng/ml (11).A study of 25-hydroxyvitamin D concentration in the resident adult population in the Catchment's area of one Birmingham hospital in the UK and other European countries has found 22% of healthy adult Asian couples (both sexes) had 25-hydroxyvitamin D less than 10 ng/ml with normal concentration in white controls (12). A recent study from Harvard in USA in June 2004 found that 24% of healthy adolescents had 25-hydroxyvitamin D levels less than 35 ng/ ml (13).The slightly decrement in vitamin D level in Iraqi population may be attributed to the malnutrition and unavailability of animal sources nutrition or insufficiency of such types of diet in Iraqi people food due to the previously imposed embargo and low income of these people, where the diet is mainly composed of carbohydrate and fiber components.The vegetarian diet of a major sector of Iraqi people adversely affects 25-hydroxyvitamin D status(14). Other animal sources are egg yolk, liver, beef, milk, cheese and fortified butter

(15). Most foods contain little or no vitamin D, so that the food is high or low in vitamin D depending on how much of that food needs to be eaten in order to maintain good health. The Recommended Dietary Allowance of vitamin D for adults is 200 IU per a day (16). Although vitamin D2 originates,from the yeast and plant sterol as ergosterol(17), vitamin D from an animal sources is still considered to be the most reliable one (15).Hence vitamin D is a fat soluble vitamin, conditions that reduce digestion or absorption of fats will decrease the ability of vitamin D to be absorbed from the intestine (18). It is important to mention that low content of vitamin D in the whole meal may contribute in decreasing serum vitamin D levels in Iraqi people, which is related to decrease in socioeconomic or other causes like the genetic factor which is likely involved in this phenomenon.

Table 3: Vitamin D Level in Serum during Seasonal Change.

Group Type	Summer					Winter					CS
		Vitamin D ng/ml			Calcium mg/dl		Vitamin D ng/ml			Calcium mg/dl	
	No.	X	SD	CV%	X±SD	No.	X	SD	CV %	X±SD	
Male	10	29.06	3.27	11.25	10.67±1.92	9	19.56	1.80	9.20	10.09±2.53	Hs
Female	10	15.80	1.73	11		11	13.06	0.52	3.99	10.01±2.03	S
Total	20	22.43	7.2	32.38	10.96±2.65	20	15.99	3.53	22.1	10.05±2.20	Hs
					10.82±2.26						

Seasonal Variation:

The results shown in table 3 reveals, through using statistical function test, that there is a highly significant ($p<0.01$) difference between the two means of 25-hydroxy vitamin D in summer and winter. The comparison was made in female groups in summer and winter seasons. The statistical analysis for the two means reveals that there is a high significant difference between them which indicate that the seasonal variation in vitamin D is evident in Iraqi population. The same comparison was made for men group and the same high significant differences were obtained. Sunlight is important to skin production of vitamin D. Environmental conditions where sunlight exposure is limited may reduce this source of vitamin D (18).The amount of vitamin D produced in the skin varies depending on the time of the day, season, and latitude (19). Hormones defined as the chemical substances that are made by one body organ to influence another. Vitamin D was originally thought to be a hormone rather than vitamin. This because vitamin D works just like hormone. Some vitamin D precursor compounds in the skin when exposed to the ultraviolet rays of the sun, they change their chemical structures and go back into the blood stream, so that sunlight exposure is the factor that influence the synthesis of vitamin D. The longer exposure to sunlight, in early morning (sun rise to 11:00 a.m.) or late after noon (3:00 p.m.) is believed to be reasonably safe (20).Geographic location and seasons of year affect vitamin D Production, In winter, people have less sun exposure and the sun is at an angle that limits the amount of ultraviolet-burn (UV-B) radiation that hits the earth. Studies have shown that people in northern climates have less vitamin D production in winter and also have bone loss (14).More UV-B photons are able to penetrate the ozone layer in the spring, and summer because the sun is directly over head. In winter, vitamin producing UV-B photons pass through the ozone layer at an oblique angle and absorbed by the ozone in greater percent (21). UV radiation is

electromagnetic radiation and part of it is UV-B, which is only a very small proportion of the sun UV radiation, the range of this band is (280-315 nm). However, UV-B can cause redness and burning, and prolonged exposure to it may cause blistering and even second degree burns, it is 1000 times more likely to cause sun burn (erythema) than UV-A which is ranging from (315-420 nm) (22).The main source of vitamin D for Australian is the exposure to sunlight. The National Health and Medical Research Council in Australia assumes that most Australians receive sufficient sunlight to more than adequately meet their vitamin D requirements (23).

Current lifestyle and work environments in developed countries, however, may be contribute to increase the prevalence of vitamin D deficiency, particularly in winter (24). Many people leave for work early in the morning, return home after dark, and drive to and from work, so that, during winter, they have limited sunlight exposure for five out of every seven days (25). In general, cutaneous synthesis provides most of vitamin D needs to the body (80%-100%) (26), and with adequate sunlight exposure dietary vitamin D may be unnecessary (27). Vitamin D stored in the adipose tissue is available during the winter, when sunlight exposure is minimal (28). Most of people, who don't allow more UV-B to reach their skin, have to take vitamin D by diet. There are many factors affect the UV-B radiation as: Window glass allows only 5% of the UV-B light range that produces vitamin D to get into home, latitude, time of year (virtually none available in winter), clouds can block UV-B light.Pollution, smog and ozone can block UV-B light, altitude (the higher up are the more UV-B light reaches), and the darker the pigmentation or more tanned the skin, the less UV-B penetrates.Mercola 2005 (29) reported that African Americans or blacks however, would need considerably more sun than whites to generate the same amount of vitamin D. An equilibriumoccurs in white skin within 20 min of ultraviolet exposure,it can take 3-6 or even to 10 times longer for pigmented skin to reach the same equilibrium point. There are significant differences in levels of vitamin D between the two groups in table (3) with seasonal variation due to high amount of sun exposure in summer in our country and the sun approximately over the head, and there are more UV-B radiation which is important in photosynthesis of vitamin D from fat under the skin or from 7-dehydrocholesterol. The decrement of vitamin D level in winter is no doubt attributable to the low sun ray in this season.

Difference between Males and Females:

The statistical comparison of serum 25-hydroxyvitamin D levels between males and females reveal that there is a high significant difference ($p<0.01$) between the two sexes .This result is shown in table 4 below.

Table 4: Vitamin D Levels in Males and Females.

Male					Female					CS
Vitamin D ng/ml				Calcium mg/dl	Vitamin D ng/ml				Calcium mg/dl	
No.	X	SD	CV%	X±SD	No.	X	SD	CV%	X±SD	
29	24.56	5.52	22.40	10.87±2.22	31	14.37	1.86	12.90	10.72±2.22	HS

Male levels are higher than females in control group. Generally there is 42.2% decline in mean serum of vitamin D level of female if compared with male. This finding was in accordance with the results that reported by many other investigators (13).The normal serum circulating 25-hydroxyvitamin D concentration in women in United States is considered to be higher than 15 ng/ml (30). However, a circulating concentration of 15ng/m is marginal for nutritional vitamin D status (31).A study from Denmark undertaken in veiled Moslem women indicates that, in the absence of sunlight exposure, they have range below 8 ng/ml of vitamin D concentration (12). Other studies assessing younger adults have reported marginal deficiency rates of 23% (10) and 43% with 8% of young women (20-39) years (24).In our country and specially our city due to social behaviors and due to religious reasons, the women must cover all the body with black cloths, and wear a veil and some times black gloves when they go outdoors. This factors act as good reason for preventing the cutaneous production of vitamin D in those women , even when they live in a sunny climate.This issue also was discussed by Taha et al., 1984 (32). They reported that "people of cultures such as Bedouins living in the Negev Desert, who are required to have most of the skin surface covered by clothing, are prone to develop vitamin D deficiency"Table 4 reveals a high variation around the mean in females compared with males, this may give an idea about the differences in nature and normal physiology of both sexes, also the hormonal changes during the normal menstruation and actual nutritional requirement for vitamin D, this variation may also account for racial factors in females (33)

References:

1-Hayes C E., Cantorna MT., Deluca HF. bone mineral content, serum vit D metabolite. Proc Soc Exp Biol Med, 1997; 216: 21-7.

2- Geer FR., Simmond SN. National Osteoporosis Foundation. Boning up on osteoporosis, hand book. 2005; 74.

3-Norman A.: Intestinal Calcium absorption, a vitamin D – hormone – mediated adaptive response. Am J Clin- Nutr. 1990; 51:290-300.

4- Brown A., Dusso A., Slatoptolsky E.: Vitamin D. Am J phys 1999; 277: 157-175.

5-Contorna MT. :Vitamin D and autoimmunity, is vitamin D status an environmental factor affecting autoimmune disease prevalence? .Proc Soc Exp Biol Med 2000; 223:230-3.

6- Holick M. F. :Vitamin D, new horizons for the 21st century. Am J Clin Nutr 1994; 60: 619 630.

7- Carl A.,Burtis FL.,Edward R.:Tietz Textbook of clinical chemistry. 3rd edition volume 3, 1999

8- Traba M., Navarro A., Marin C., et al.: A comparative study of 25-hydroxyvitamin D3 separation in human serum. In: Vitamin D chemical. Biochemical and clinical Endocrinology of calcium metabolism. A.W. Norman K, Schaefer. D.V. Herrath. H.G. Grigoleit. Eds. New York, Walter De Gryter. 1982; pp. 805-807.

9- Holick MF. :Calcium and vitamin D. Diagnostics and Therapeutics. Clin Lab Med. 2000; 20 (3):569-90.

10- MacGrath J., Kimlin M., Saha S., et al.:Vitamin D insufficiency in South-east Queensland. Med J Aust. 2001; 174: 150-151.

11- Yamashita H., Noguchi S., Takatsu K., et al.: High prevalenceof vitamin D deficiency in Japans
female patients with Graves diseases. Endocr. J. 2001; 48(6): 63-9.
12- Shaw N J.,Pal B R. :Vitamin D deficiency in UK Asian families , Am J Phys. 2001;17;212-7
13-Joseph M. , John C. Measuring our vit D levels. Endocr. J.2005; 17;518-23.
14- Erin P. R., Jeffrey P., Nutritional News. Clin Nutri services 1998.
15- Elizabeth M.R.,. :The essential Guide to vitamins and minerals 2nd Ed 2001.
16- Yagya. A. P. Vitamin D deficiency. J.Bone Miner Metab.2005;174:23-29.
17- Holick H. L. :Biochemistry and physiology-Aspect of Nutrition. 4th Ed. Mosbey 2004.
18-Jhon G R. competitive protein binding redio assay for 25(OH) D3. J.Clin. Endocr.Metab.1971;33:992-5.
19- National Osteoporosis Foundation. Osteoporosis prevention-calcium and vitamin D recommendations. handbook. Washington, D.C.2005;74-
20- Cooken E.,Haddad JG.vit D binding protein (globulin). Endocr. Rev.1989;10:294-9
21- Harris BS, Bunket JW. Vit D potency of human breast milk. Am J public health. 1999;29:744-7
22- General Ultraviolet Health Effect Information. The Environmental Health Center. Sun safety
site and sun protection, path finder science2004.
23- National Health and Medical Research Council. Recommended dietary intakes for use in Australia, Canberra:AGPS, 1991.
24- Pascoe J., Henry M., Nicholson G., et al. :Vitamin D status.J Nutri. 1981; 111:1240-8.
25- Kim J., Moon S. :Time spent outdoors and seasonal variation in serum concentration of 25-
hydroxyvitamin D in Korean women. Int J Food Sci 2000; 51:439-451.
26- Glerup H., Mikkelsen K., Poulsen L., et al. :Commonly recommended daily intake of vitamin
D is not sufficient if sunlight exposure is limited. J Intern Med 2000; 247: 260- 268.
27- Holick M.: Sunlight “Dilemma: risk of skin cancer or bone disease and muscle weakness. Lancet 2001; 357: 4-6.
28- Reeve L E. Food and nutrition board. Dietary reference intakes for Ca, P, Mg, Vitamin D and
F. Washington: National Academy Press, 1997.
29- Goseph M. : test values and treatment for vitamin D deficiency. J Biol Chem. 1992;53:219-23
٣٠- Favus M. Metabolic bone disease and disorders of mineral. metabolism. 4th ed .Newyork: lippincott, 1999: 467-70
31- Hollis B W. :Assessment of vitamin D nutritional and hormonal status
Tissue Int.1996;58:4-5.
32- Taha S.A., Dost S.M., Sedrani S. H.: 25- Hydroxyvitamin D and total calcium . Extraordinarily
low plasma concentrations in Saudi mothers and their neonates. Pediatr, Res 1984; 18 : 739-
741.
33- Bruce W., Hollis., Carol L., et al. :Assessment of dietary vitamin D requirements during pregnancy and lactation Medical University of South Carolina, 2003.