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RESEARCH ARTICLE

STATUS OF SERUM 25 HYDROXYVITAMIN D AND THE PREVALENCE OF ITS DEFICIENCY IN IRAQI HEALTHY MEN: AGE DEPENDENT STUDY

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ABSTRACT

Background: This study was designed to assess the status of vitamin D concentration (deficiency and insufficiency), by measurement of 25 hydroxyvitamin D 25(OH)D in Iraqi healthy men and to show the effect of age on it.

Subjects and methods: This cross-sectional study was carried out at Biochemistry Department, College of Medicine, University of Baghdad, Iraq, during the period from January 2013 to November 2013. This study included 180 healthy men, aged 18-82 years encountered from different regions of Iraq. Men were classified into several groups according to their ages; group VAI (GVAI; < 50 year), group VAII (GVAII; > 50 year), group VBI (GVBI; < 60 year) and group VBII (GVBII; > 60 year). High performance liquid chromatography technique (HPLC) was used for the determination of serum 25(OH)D.

Results: The mean value of 25(OH)D concentrations in Iraqi health men was 27.77±12.18 ng/ml, with a range of 1.60-66.89 ng/ml. The results revealed that 28.90 % of the studied men had vitamin D deficiency (< 20 ng/ml), 35.0 % had vitamin D insufficiency (>20<30 ng/ml), and 36.10 % had normal D (> 30 ng/ml). The mean of serum 25(OH)D of men with age ≥ 60 year was significantly lower than that of men of age < 60 year (p=0.038). There was significant positive correlation between the values of age and the serum concentrations of 25(OH)D in group age (40-<60 year) with (r=0.224, p=0.026).

Conclusion: The present study revealed the relatively high prevalence of both deficiency and insufficiency of serum 25(OH)D in Iraqi healthy men. The elderly men of age of ≥60 year are the more obvious ones who suffered from D deficiency and who are of urgent need for this vitamin supplementation to combat its complications.

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INTRODUCTION

Vitamin D is a cholesterol derived, fat soluble, steroid substance present in the body as either ergocalciferol (D 2) or cholecalciferol (D3) (Matthew Smith, 2010). The majority of vitamin D is produced in vivo as D3 when skin is exposed to the ultraviolet B wavelength of sunlight and this pathway produces more than 90% of the body's vitamin D (Endres and Rude, 2006; Norval *et al*., 2007). This pathway involved the photoisomerization of 7-dehydrocholesterol to form inactive previtamin D3 during exposure to UVB radiation (Webb, 2006). Previtamin D3 then undergoes activation process through its firstly hydroxylation in the liver, resulting in the formation of 25-hydroxyvitamin D3 [25(OH)D3], the primary (predominant) circulatory form. The second step of activation involved the subsequent hydroxylation of 25(OH)D3 in the kidney to yield the biologically active form of vitamin D, calcitriol [1,25-(OH)2D] (Lu *et al*., 2012). A minority of vitamin D is absorbed in the gut from food products as D2/D3. Very few foods naturally contain vitamin D, and foods that are fortified with vitamin D are often inadequate to satisfy either a child's or an adult's vitamin D requirement (Holick and Chen, 2008). Vitamin D is an important component in many bodily processes from molecular to systemic roles and is different from other vitamins in that it may be described as a hormone. Pathologies traditionally associated with a loss of vitamin D homeostasis are due to hypercalcaemia (hypervitaminosis D) or hypocalcaemia (hypovitaminosis D), which causes rickets and osteomalacia (Carmeliet *et al*., 2003; Lips, 2004).

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Vitamin D is known to be an essential element for bone metabolism and skeletal health. Recent studies increasing interest in the influence of vitamin D on extra skeletal health. It has been reviewed that vitamin D status may play a role in metabolic syndrome, insulin resistance and diabetes mellitus type I and II, tumor carcinoma, multiple sclerosis and other autoimmune diseases, and ischemic heart diseases (Lu *et al*., 2012). The serum level of 25(OH) D is a reliable indicator of the vitamin D status of an individual (Lips, 2004). Although vitamin D deficiency has been defined, there is no consensus on a cut-off level for defining vitamin D deficiency. Nevertheless, it is widely suggested that the circulating 25(OH)D concentrations of less than 30 ng/mL should be considered as indicator of vitamin D insufficiency, whereas circulating concentrations of less than 20 ng/mL should be considered as vitamin D deficiency (Ali *et al*., 2009). Using these definitions, it has been estimated that between 40% and 100% of elderly US and European non-hospitalized men and women are either vitamin D deficient or insufficient (Holick, 2007). High rates of vitamin D deficiency have been reported in children and adults worldwide (Holick, 2008). In the sunny countries, numerous studies have demonstrated a high prevalence of low vitamin D status. About 25–50% of children and adults in Saudi Arabia, Qatar, India, and Lebanon had vitamin D deficiency (Ali *et al*., 2009; Badawi *et al*., 2012; Holick, 2007; Fields *et al*., 2011; Hoteit *et al*., 2014). Wat *et al*. have shown that southern Chinese adults exhibit high prevalence (62.8%) of vitamin D insuffficiency in association with secondary hyperparathyroidism (Wat *et al*., 2007).

Subjects and methods

This cross-sectional study was carried out at Biochemistry Department, College of Medicine, University of Baghdad and at Baghdad Teaching Hospital, Iraq, during the period from January 2013 to November 2013. This study included 180 healthy men, aged 18-82 years encountered from different regions of Iraq. All men were free from any acute illness or known history of diabetes mellitus and other metabolic disorders, different bone diseases, liver diseases, renal diseases, thyroid and other endocrinology disorders, ischemic heart diseases, autoimmune and immunologic disorders, neurologic and hematologic disorders. Men were classified into three groups according to their ages; Group I (GI) consisted of 21men, aged 22-<40 year, group II (GII) included 99 men, aged 40-<60 year, and group III (GIII) involved 60 men who aged 60 year and more. These men were also subdivided according to their age into; group VAI (GVAI) aged of less than 50 year (n=94), and group VAII (GVAII) aged of ≥ 50 year (n=86). Another classification depending on the same factor, the age, included group VBI (GVBI) with age of less than 60 year (n=120) and group VBII (GVBII) with age of ≥ 60 year (n=60). Three to five milliliter (ml) of venous blood sample was collected from peripheral vein of each men. The aspirated blood was transferred to plain test tube, left to clot for 15 minute and then centrifuged for 10 minutes at 3000 rpm. The clear serum was stored at -20 °C until used for measurement the concentrations of 25-hydroxyvitamin D [25(OHD)]. The use of 25(OH)D concentrations as a biomarker of vitamin D status is documented. There is no absolute consensus on the cut-off value between a normal and low level of vitamin D, but most experts now recommend the normal level of 25(OHD) to be 30 ng/ml or more. They have also agreed to define vitamin D insufficiency as a level between 20-<30 ng/ml and deficiency when level is less than 20 ng/ml. (Ali *et al*., 2009; Naeem *et al*., 2011). The high performance liquid chromatography technique (HPLC) was used for the determination of serum 25(OH)D. Vitamin D, the fat soluble vitamin, was separated on NH_2 column isocratically after obtaining optimum conditions (temperature, flow rate, eluent composition). The eluent used was: tetrahydrofuran (THF), Methanol (MeOH), water (H_2O; HPLC grade) in proportion: 70: 20: 10: V/V, respectively. The flow rate was: 1 ml / min, temperature: room temperature and detection was UV 285 nm.

Formal consent was taken from each subject. We received ethical approval from the Scientific Committee of the Biochemistry Department, College of Medicine, University of Baghdad,Iraq. SPSS version 6 for window was used for all statistical analyses. Statistical significance was assessed by ANOVA, and student t-tests. The linear regression test was applied for the correlation between different parameters and the significance of the p-value was checked using t-test. P-values of less than (0.05) were considered significant.

RESULTS

Table 1 shows the mean (±SD) values of age and the serum concentrations of 25(OH)D of the whole subjects group. The mean of age was found to be 50.96±13.32 year, with a range of 22-82 year and the mean value of 25(OH)D concentration was 27.77±12.18 ng/ml, with a range of 1.60-66.89 ng/ml. The percentage of low vitamin D as assessed by measurement of serum 25(OH)D in Iraqi healthy men was illustrated in table 2. The results revealed that 52 out of the studied 180 men had vitamin D deficiency (< 20 ng/ml) with percentage of 28.90 %, 63 men (35.0 %) had vitamin D insufficiency (>20<30 ng/ml), and 65 of the involved 180 men (36.10 %) had normal D (> 30 ng/ml).

Table 1. Mean (±SD) Values of Age and Serum Concentrations of 25(OH) D of the Whole Men Group

Parameter	(Mean± SD)	Range
Age (year)	(50.96± 13.32)	(22-82)
25(OH)D (ng/ml)	(27.77± 12.18)	(1.6-66.89)

Table 2. Percentage of 25(OH)D Deficiency, Insufficiency and Normal Values in Iraqi Healthy Men

Parameter		No.	Percentage
25(OH)D Deficiency	(< 20 ng/ml)	52	28.90 %
25(OH)D Insufficiency	(>20 -<30ng/ml)	63	35.0 %
25(OH)D Normal	(> 30 ng/ml)	65	36.10 %
Total		180	100.0 %

Table 3. Mean (±SD) Values of Serum Concentrations of 25(OH)D of GI, GII, and GIII

Parameter	GI (22- <40 year) (n=21)	GII (40- <60 year) (n=99)	GIII (≥60 year) (n=60)
25(OH)D^{NS}	27.14± 10.21	29.51± 11.94	25.11± 12.87
(ng/ml) Range	9.87-58.88	1.6-61.33	4.23-66.89

NS non significant differences among groups

Table 4. Mean (±SD) Values of Serum Concentrations of 25(OH)D of GVAI, GVAII, GVBI, and GVBII

parameter	GVAI (<50 year; n=94)	GVAII (≥50 year; n=86)	GVBI (<60 year; n=120)	GVCBII (≥60 year; n=60)
25(OH)D(ng/ml)	27.76± 10.54^{NS}	27.77± 13.82	29.09± 11.65▲	25.11± 12.87

▲t-test revealed significant difference between GVBI and GVBII (p=0.038)
NS non significant difference between GVAI and GVAII

Table 3 shows the mean (±SD) values of serum concentrations of 25(OH)D in healthy men of the three groups GI (27.14±10.21 ng/ml), GII (29.51±11.94 ng/ml) and GIII (25.11±12.87 ng/ml), with no significant differences. The mean (±SD) values of serum concentrations of 25(OH)D of GVAI and GVAII as well as of GVBI and GVBII were presented in table 4. The mean of 25(OH)D levels of men with age of more than 60 year (GVBII; 25.11±12.87 ng/ml) was significantly lower than that of men of age of less than 60 year (GVBI; 29.09±11.65 ng/ml; p=0.038). However, the mean value of 25(OH)D concentrations of men of less than 50 year (GVAI; 27.76±10.54 ng/ml) did not differ significantly from those of age of more than 50 year (GVAII; 27.77±13.82 ng/ml). There was significant positive correlation between the values of age and the serum concentrations of 25(OH)D in group II (40-<60 year) with (r=0.224, p=0.026). However, there was no significant correlation between the age and 25(OH)D of the entire group of the studied men as well as among the GI and GIII.

DISCUSSION

The incidence of both vitamin D deficiency and insufficiency in Iraqi healthy men was relatively high 28.90 % and 35 %, respectively. Prevalence of low vitamin D status in Pakistanis residing in Karachi was surprisingly high; 186 subjects of the study population (n=233; 76.2%) had deficiency of vitamin D, 36 (14.80 %) had insufficiency and 22 (9%) had normal level of vitamin D, despite abundant sunlight throughout the year in this region (Mahmood *et al*., 2009). These authors concluded that men avoid sun exposure due to high temperature in summer season in Pakistanis in addition to their misconception regarding unawareness regarding the source of Vitamin D. Effect of sunlight on vitamin D status has been well documented and confirms the importance of sunlight exposure in the synthesis of vitamin D (Saraiva *et al*., 2005). Area of skin exposed and duration of sunlight exposure strongly correlated with vitamin D levels (Meddeb *et al*., 2005). As dark skin requires more sun exposure than less pigmented skin to produce similar amount of vitamin D. (Saraiva *et al*., 2005) Dietary factor was another determinant of the study regarding high prevalence as most of the participant were consuming low amount of vitamin D rich food (Mahmood *et al*., 2009; Saraiva *et al*., 2005). In a study conducted in the eastern region of Saudi Arabia on 100 healthy men, aged 25 to 35 years was found that 28% had a low level of 25(OH)D, of whom 18% were insufficient and 10% were deficient. Also, the same study found that 37% of the men aged 50 years and older were suffering from low vitamin D levels (insufficiency and deficiency). (Ali *et al*., 2009) However, another study conducted on180 healthy individuals aged 19-72 years from Qassim region, Saudi Arabia, indicated that 28.3% were having deficient vitamin D level and 39.4% were insufficient (Naeem *et al*., 2011). The authors concluded that deficiency of vitamin D might be related to lack of adequate exposure to sunlight and possibly inadequate consumption of dairy products. They believed that there is an urgent need for public education about the role of vitamin D in health to avoid the complications of vitamin D insufficiency and deficiency.

The recommendations of these researchers involved the public education and awareness about the importance of sparing time for sun exposure for the sake of obtaining vitamin D, health education at Primary Health Care centers regarding consumption of diet rich in vitamin D as fish, liver, cheese, and fortified milk and so on, should be emphasized. Also, these studies indicated that sunlight exposure of arms and legs for 30 minutes without sunscreen to could provide daily need of vitamin D to the human body. A study conducted on Lebanese healthy individuals, the prevalence of vitamin 25(OH)D deficiency was found to be ranged between 44% and 60% and that of insufficiency was 78% (Hoteit *et al*., 2014). The results of the study carried out in Isfahan, a sunny city located in the central part of Iran, confirmed the high prevalence of vitamin D deficiency (50.8%) and insufficiency (19.6%) among the apparently healthy Iranian population aged 20-80 years.

The findings showed that women were more likely to be vitamin D-deficient than men, especially for severe vitamin D deficiency. However, there was no significant difference in vitamin D insufficiency between men and women. Although Isfahan is a sunny city, direct exposure to sun is, however, limited. Most men wear long-sleeve shirts, especially those who work in governmental administrations. Fear from skin cancer encourages people to use anti-solar creams on their face. Living in apartments which is increasing due to increased population of the country and tendency to live in big cities are among other factors which restrict exposure to sun in Iran (Hovsepian *et al*., 2011). The results of another study also conducted on Iranian healthy subjects revealed that the rates of deficiency and insufficiency of 25(OH)D levels were found to be 47.0% and 24.0%, respectively. It seems that factors, such as style of clothing, air pollution, skin pigmentation, and insufficient vitamin D intake, lack of routine enrichment of foods with vitamin D in Iran, could be responsible for these findings (Hovsepian *et al*., 2011; Moussavi *et al*., 2005).

These authors recommend fortification of foods with vitamin D to treat and prevent vitamin D deficiency as the styles of clothing. However, in a study conducted on Jordanian population found high rate of low vitamin D status in adult women (37.3%), which contrasts markedly with a relatively low rate in adult men (5.1%). However, the majority of the involved employers of this study were of age of less than 40 years, and those of age of more than 60 years were only 12% of the total involved population (Batieha, *et al*., 2011), which may interpret the discrepancy from that found in Iraqi population and other neighboring sunny countries. Vitamin D deficiency is nearly universal above the age of 50 years in northern part of India (Marwaha *et al*., 2011). The study highlights the inadequacy of a daily vitamin D intake (200-400 IU) in normalizing serum 25(OH)D levels in this population, and a daily intake of (2000 IU) of cholecalciferol would be required for this. Similarly, among healthy adults men living in Shanghai, vitamin D deficiency/insufficiency is very common (30% and 84%, respectively). Low serum 25(OH)D levels were associated with both secondary hyperparathyroidism and high bone turnover status; the decreases in 25(OH)D concentration below 20 ng/ mL will result in rapid increase in PTH. The authors strongly recommended the taking of calcium and vitamin D supplements when the serum 25(OH)D concentration is below 20 ng/ml. In addition, for the appropriate maintenance of bone turnover, a level of serum 25(OH)D of 30 ng/ml is suggested (Lu *et al*., 2012).

In conclusion, the present study revealed a relatively high prevalence of both deficiency and insufficiency of serum 25(OH)D concentration in Iraqi healthy men and the elderly men of age of ≥60 year are the more obvious ones who suffered from vitamin D deficiency and who are of urgent need for this vitamin supplementation to prevent its complications. The study recommended public education and awareness about the deficiency of vitamin D and the importance of sun exposure and health education regarding consumption of diet rich in vitamin D as fish, liver, cheese, and fortified food. Future studies are considered to evaluate the status of vitamin D in Iraqi healthy women and to show the effect of physiological factors like pregnancy, lactation and menstruation on it.

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