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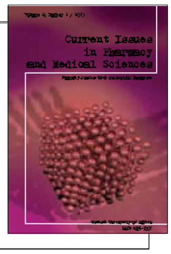

Blood serum Vitamin D status evaluated in healthy people

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ABSTRACT

The purpose of the study was to evaluate the status of vitamin D in healthy subjects. Vitamin D level was determined on the basis of blood serum 25(OH)D concentrations, taking into account the time of blood collection and age. Moreover, it was to find correlations between serum concentrations of: 25(OH)D, calcium, inorganic phosphates and serum alkaline phosphatase (ALP) activity. The randomized study was carried out on a group of 40 healthy subjects. Each person had venous blood samples drawn twice: from 6 March, 2012 to 15 March, 2012 and from 25 June, 2012 to 6 July, 2012. The results found the majority of subjects had lowered serum 25(OH)D concentrations compared to the reference range determined in the winter months of little insolation and in the summer months of much insolation. In the summer the blood serum concentration of 25(OH)D and ALP activity were statistically significantly higher in comparison with the winter and spring. The concentration of 25(OH)D was not statistically significantly related to age, concentrations of Ca, inorganic phosphates and ALP activity.

Keywords: Vitamin D, calcium, inorganic phosphates, ALP, healthy humans serum

INTRODUCTION

Vitamin D has been known to mankind since 1820. The term "vitamin D" (calciferol) defines two chemicals from a group of 9,10-secosteroids: ergocalciferol and cholecalciferol. Regulation of calcium-phosphate metabolism is the most important function of vitamin D. The final effect of 1,25$(OH)_2$D (1,25-dihydroxycholecalciferol) is to increase blood calcium and phosphate concentrations to ensure optimal conditions for bone mineralization [11,17]. Vitamin D deficiency results in disordered bone mineralization which leads to rickets in children and osteomalacia in adults. In rickets, the bones, being loaded by the increased body weight of a growing child, become deformed and bent [8]. For many years, researchers believed that calcium-phosphate metabolism regulation and involvement in bone tissue metabolism was the only function of vitamin D. The discovery of pleiotropic activity of vitamin D allowed understanding better its role in the body. At present, it is known that vitamin D deficiency contributes to many chronic conditions, including cardio-vascular and neoplastic problems, diseases due to autoaggression, metabolic syndrome and obesity [12,20].

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Full clinical picture of vitamin D deficit is usually preceded by a period of subclinical manifestations related to gradual decrease in blood 25(OH)D (25-hydroxycholecalciferol) concentration [11,14].

The purpose of the study was to evaluate vitamin D status in healthy subjects. Vitamin D level was determined on the basis of blood serum 25(OH)D concentration taking into account age. Moreover it was to determine the frequency of vitamin D deficits on the basis of blood serum 25(OH)D results and to find correlations between blood serum 25(OH)D and the concentrations of calcium, inorganic phosphates and serum alkaline phosphatase (ALP) activity.

MATERIAL AND METHODS

A randomized study was carried out on a group of 40 healthy subjects aged 22-63 years. To obtain blood serum each person had venous blood samples drawn fasting in the morning, twice: from 6 March, 2012 to 15 March, 2012 and from 25 June, 2012 to 6 July, 2012. The concentrations of 25(OH)D, calcium, inorganic phosphates and serum ALP activity were determined in blood serum. The concentration of 25(OH)D was measured on COBAS analyzer *e411* (Roche) by electrochemiluminescence (ELC) method. The concentrations of calcium, inorganic phosphates and ALP activity were determined by COBAS *INTEGRA 400 Plus* (Roche). Calcium concentrations were determined by ortho-cresolphthalein complexone (OCP) colorimetric method. The concentrations of inorganic phosphates were measured using the method based on the reaction with ammonium heptamolybdate. The activity of ALP was evaluated by a standardized method using optimal substrate concentrations and 2-amino-2methyl-1-propanol buffer with magnesium and zinc ions (Roche Diagnostics). All biochemical tests were conducted in one-series session at The Medical University Hospital Diagnostic Laboratory.

RESULTS

The results found significantly increased serum ALP activity and 25(OH)D concentration obtained in the second series compared to the first collection. Other tested parameters did not show significant differences in the concentration values of calcium and inorganic phosphates between the first and second series (Table 1, Fig. 1).

Table 1. Descriptive statistics of the concentrations of 25(OH)D, calcium, inorganic phosphates and ALP activity with reference to the time of blood sample collection

Parameter	First collection (c1)			Second collection (c2)		
	$\bar{x}$±SD	Me	25–75%	$\bar{x}$±SD	Me	25–75%
25(OH)D (ng/ml)	15.62±7.25	15.67	10.81–19.43	20.78±7.23*	20.44	14.89–25.95
Ca (mmol/l)	2.25±0.30	2.32	2.26–2.38	2.30±0.09	2.33	2.25–2.37
P (mmol/l)	1.08±0.22	1.09	0.96–1.25	1.15±0.26	1.08	0.96–1.30
ALP (U/l)	55.55±19.31	54	45–65	62.58±19.44*	58	50–76

$\bar{x}$ – arithmetic mean; SD- standard deviation; Me- median; 25–75% – percentile range; 25(OH)D – 25-hydroxycholecalciferol concentration; Ca – calcium concentration; P – inorganic phosphates concentration; ALP – alkaline phosphatase activity; * p<0.001

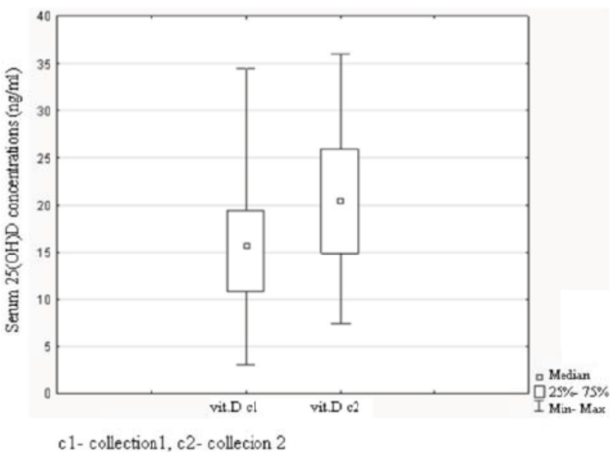

Fig. 1. Serum 25(OH)D determined on the first and second sample collection

Table 2 presents the frequency of particular concentration ranges of serum 25(OH)D with reference to the collection series. During first collection (c1) the frequency of vitamin D deficit, defined as serum 25(OH)D concentration range of 0-10 ng/ml, was 22.5%, the frequency of 25(OH)D insufficiency within the range of 10-20 ng/ml was 52.5%, the frequency of hypovitaminosis D (25(OH)D concentration range of 20-30 ng/ml) was 20% and optimal level (25(OH)D concentration range of >30 ng/ml) was 5%. During second collection (c2) the respective values for deficits (both ranges: 0-10 ng/ml and 10-20 ng/ml) were 5% and 42.5%, hypovitaminosis D – 42.5% and optimal level – 10%.

Table 2. Frequency of serum 25(OH)D concentrations determined during the first (c1) and second (c2) collection of blood

	Serum 25(OH)D concentrations (ng/ml)	Frequency of occurrence in the examined population	
		First colletion (c1)	Second collecion (c2)
Vit. D deficit	0–10	22.5%	5.0%
Vit. D insufficiency	10–20	52.5%	42.5%
Hypovitaminosis D	20–30	20.0%	42.5%
Vit. D recommended level	30–80	5.0%	10.0%

Moreover, there were no statistically significant differences between serum 25(OH)D concentrations determined during the first and second collection and other parameters determined in both series. No correlations were found among the studied parameters (Table 3).

Table 3. Correlations between selected parameters

Parameters		Number of examined samples n=40	
		Correlation (R Spearman)	Significance p
25(OH)D (ng/ml) First collection (c1)	Ca c1 (mmol/l)	0.275	0.086
	P c1 (mmol/l)	0.046	0.776
	ALP c1 (U/l)	-0.159	0.327
	Age (years)	-0.075	0.644
25(OH)D (ng/ml) Second collection (c2)	Ca c2 (mmol/l)	0.176	0.276
	P c2 (mmol/l)	-0.267	0.095
	ALP c2 (U/l)	-0.106	0.516
	Age (years)	-0.139	0.391

DISCUSSION

Literature data suggest that hypovitaminosis D seems to be epidemic in many countries all over the world, including Poland. It is considered one of the major health problems in humans. The problem seems to be alarming since, according to the present knowledge, normal content of vitamin D is not only indispensable for its calcemic activity but also for vitamin D influence on the cardiovascular system, immune system, CNS, neoplastic diseases, dermatological ailments, metabolic syndrome and type 2 diabetes [4-6,15].

Our results revealed that mean concentration of serum 25(OH)D determined in summer was statistically significantly higher (p<0.001) in comparison to the mean value obtained in winter and spring. Similarly, Hill et al. [9], who examined 76 postmenopausal women from Ireland, observed distinct fluctuations of vitamin D metabolites depending on the season of the year. They found that 25(OH)D concentrations were substantially higher in the summer months compared to the winter season. In another study, Pasco et al. [18] examined 3280 females aged over 55 years from Australia. They observed maximum serum 25(OH)D concentration in the summer and minimum values in the winter and noticed that lowered 25(OH)D concentrations correlated with increased markers of bone resorption. Also Bhattoa et al. [2] focused on seasonal fluctuations of serum 25(OH)D. They examined 319 postmenopausal females from Hungary. They found that serum 25(OH)D level depended on the mean number of sunlight hours during three months prior to blood collection, age and dietary calcium content [19,20].

Our study focused on the frequency of particular vitamin D concentrations depending on the time of blood sample collection. The frequency of particular vitamin D concentrations in Polish population was analysed by Napiórkowska et al. [16]. They examined vitamin D status in a group of 274 elderly women aged 69.1±5.7 years mean in Poland. In the winter mean serum 25(OH)D concentration was 13.6 ng/ml; optimal 25(OH)D concentration (>30 ng/ml) was found in 4% examined women, 25(OH)D insufficiency (20-30 ng/ml) was determined in 12.8% examined and deficit value of >20 ng/ml was observed in 83.2% women. Hypponen and Power [10] also evaluated the frequency of particular vitamin D concentrations. They examined 7500 citizens from Great Britain and obtained slightly different results, using similar cut-off values. In the winter-spring months they found the following results: the frequency of vitamin D deficit (defined as serum 25(OH)D concentration range of 0-10 ng/ml) was 15.5%; the frequency of 25(OH)D insufficiency (within the range of 10-16 ng/ml) was 31.1%; the frequency of hypovitaminosis D (25(OH)D (concentration range of 16-30 ng/ml) was 40.5% and optimal level (25(OH)D concentration range of >30 ng/ml) was 12.2%, while in the summer months the respective values for deficit were 3.2%, insufficiency – 12.2%, hypovitaminosis D – 42.5% and optimal level - 10%.

In this study we also analyzed the correlation between serum 25(OH)D concentrations and basic parameters of calcium-phosphate metabolism (concentrations of calcium, inorganic phosphates and ALP activity). However, no significant correlations between those parameters were found. Kashi et al. [13], who investigated a group of 351 people from Sari, northern Iran obtained similar results. They did not find significant correlations between serum 25(OH)D, calcium, inorganic phosphates concentrations, and ALP activity. Similarly to us, they found significantly higher ALP activity in the summer compared to winter. Among the population of Sari the frequency of vitamin D deficit (defined as 25(OH)D concentration >30 ng/ml] was 90.6% in the summer compared to the winter season when it was 93.2%. Our results revealed that 90% examined in the second test and 95% in the first series had serum 25(OH)D concentrations >30 ng/ml.

We did not find significant correlations between serum 25(OH)D concentrations and age. This fact is confirmed by literature data. Fassi et al. [7] did not find correlations with age. They examined serum 25(OH)D concentrations in 159 females at various age, citizens from Buenos Aires. In the winter vitamin D deficit was found in 15.9% of young women and in 14.2% of elderly women. In the summer the concentrations of 25(OH)D were increased in both groups. Also Andersen et al. [1] evaluated 25(OH)D concentrations in teenage girls and elderly women from Poland, Denmark, Ireland and Finland. Concentration of 25(OH)D ng/ml was noted in 37% of teenage girls and 17% of elderly women, concentration of 25(OH)D >20 ng/ml was found in 92% and 37% respectively. Vitamin D level positively correlated with vitamin D supplementation and hours of exposure to sunlight [3,13,16].

CONCLUSIONS

To sum up, we need to state that in the examined group of healthy people vitamin D concentrations, expressed as blood serum 25(OH)D concentrations, are lower in the winter compared to the reference values. The results obtained during the study carried out from 25 June, 2012 to 6 July, 2012 were statistically significantly increased compared to the results of measurements from 6 March, 2012 to 15 March, 2012. The results showed that vitamin D status does not depend on age. No correlation was found between vitamin D concentrations expressed as serum 25(OH)D and the concentrations of calcium, inorganic phosphates and ALP activity. Seasonal fluctuations in vitamin D contents, their clinical consequences as well as possible benefits of vitamin D supplementation in the period of little insolation need further examination.

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