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Vitamin D Status and Its Relationship with Age, Gender and Parathyroid Hormone

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Abstract: Vitamin D is essential for our health. It plays a crucial role in bone mineralization through calcium and phosphate metabolism. Abnormal levels of vitamin D are linked to a group of different diseases, such as rickets in children, osteomalacia in adults, muscle problems, malfunctioning of the immune system, etc. From previous studies on vitamin D status in the Albanian population, has resulted that vitamin D levels are low to insufficient, although Albania is a sunny country. Taking into consideration this fact, this paper aims to assess the situation of vitamin D in a group of adult individuals, randomly selected in the district of Tirana. Vitamin D status is studied and evaluated based on the comparison of two randomly selected groups, in a difference of time for a decade (from 2009-2010 to 2019-2020). Data analysis is based on the comparison of vitamin D levels between genders and different age groups, as well as on the comparison of vitamin D level between healthy individuals and those with parathyroid problems. For the purpose of this study, a 10 ml fasting blood sample was collected in pre-chilled tubes from each subject and has been analyzed based on an immunoenzymatic method, using ELFA (Enzyme-Linked Floureshence Assay) technique, by VIDAS system. Further statistical analysis of the data collected is done by SPPS 20 program for windows. Comparing the findings of this study with those from the previous studies, as a conclusion we can say that the situation of vitamin D level appears improved to the previous years, but still the level of insufficiency is high.

Keywords: Vitamin D, Parathyroid hormone, calcium metabolism, Albanian population

Introduction

Vitamin D is a fat soluble organic compound, acting like a hormone and playing a crucial role in calcium and phosphate homeostasis in the human body. Vitamin D and its' metabolites are a crucial part of the endocrine system that controls whole body calcium homeostasis. The goal of this hormonal control is to regulate serum calcium levels so that they are maintained within a very narrow range. Both vitamin D and parathyroid hormone are essential in calcium metabolism. Through this crucial function, vitamin D is essential and is involved in many physiological processes in our body, such as normal bone mineralization, nerve conduction and normal functioning of nervous and muscular system and other immuno-modulatory cascades.

Vitamin D comes in two forms (D_2 and D_3) which differ chemically in their side chains. These structural differences alter their binding to the carrier protein vitamin D binding protein (DBP) and their metabolism, but in general the biologic activity of their active metabolites is comparable (Bikle, 2017). 1α,25-Dihydroxvitamin D_3 ($1,25(OH)_2D_3$) is the hormonally active form of vitamin D_3 (Rochel & Molnár, 2017). Its synthesis and its metabolites, their transport and elimination as well as action on transcriptional regulation involves the harmonic

cooperation of diverse proteins with vitamin D binding capacities such as vitamin D binding protein (DBP), cytochrome P450 enzymes or the nuclear vitamin receptor (VDR) (Rochel & Molnár, 2017).

Vitamin D (VD) deficiency is a health condition that affects an important proportion of the worldwide population (Roth et al., 2018). Severe vitamin D deficiency may cause rickets in infants or children and osteomalacia in adults, though it is now uncommon in developed countries (Chang & Lee, 2019). It is well-known that vitamin D can be obtained by sun exposure or limited natural dietary sources. According to Passeron et al. (2019) terrestrial ultraviolet radiation (UVR) is the main determinant of vitamin D status (Passeron et.al., 2019). Multiple intrinsic and extrinsic factors modulate vitamin D synthesis and overall status, including genetic polymorphisms, age, geographical location, sun exposure behaviour, UVB dose, clothing, body surface area (BSA) exposed (Rochel & Molnár, 2017; Cabalín et al., 2021). Sun induced vitamin D synthesis is greatly influenced by season, time of day, latitude, altitude, air pollution, skin pigmentation, sunscreen use, passing through glass and plastic, and aging (Wacker & Holick, 2013). Other studies on the effect of sunscreen use mention that sunscreen use for daily and recreational photoprotection does not compromise vitamin D synthesis, even when applied under optimal conditions (Passeron et al., 2019). Several studies about seasonal variation analyses demonstrated that serum 25(OH)D levels were lower in winter than in summer in high latitudes (Greene-Finestone et al., 2011; Klingberg et al., 2015). The American Academy of Dermatology declared ultraviolet radiation to be a known skin carcinogen, so it may not be safe or efficient to obtain vitamin D via sun exposure or other artificial sources (Chang & Lee, 2019). Although vitamin D can be directly taken from food sources, such as dairy products, fishes, and oils, a major portion of the body's vitamin D is synthesized de novo via exposure of skin to sunlight (UVB rays), resulting in cholecalciferol (vitamin D3), which is further converted to 25-hydroxycholecalciferol [25(OH)D], in the liver (Malik et al., 2020).

After all, taking into consideration the undisputed effect of sun exposure in vitamin D synthesis and the fact that Albania is a country with an average of 300 hundred sunny days, this paper aims the assessment of vitamin D status and its relationship with age, gender and parathyroid hormone, in a compared way between two groups of adult individuals, randomly selected in the district of Tirana in two different periods of time; the first group from 2009-2010 and the second one from 2019-2020.

Method

A group of 288 adult individuals randomly chosen from 20 to 83 years old, was included in this study (73.6% of the group are females and 26.4% are males). This study aims the assessment of vitamin D status in two different groups in two different periods of time, in a compared way. In the first group were included 129 individuals, tested for vitamin D levels during the period of time from 2009-2010 and in the second group were included 159 individuals, tested for vitamin D levels during the period of time from 2019-2020. The objectives of our study were as followed:

1-Assesment of vitamin D in our sample in a compared way in two different periods of time, in two randomly chosen groups (the first group from 2009-2010 and the second one from 2019-2020)
2-Analysis of the results; comparison of vitamin D level based on the gender difference and age-group
3- Comparison of vitamin D level between a group of healthy individuals and another group with parathyroid problems

For the purpose of this study, a 10 ml fasting blood sample was collected in pre-chilled tubes with anticoagulants (lithium-heparin and not EDTA) from each subject. Vitamin D assessment of these samples was dane based on an immunoenzymatic method, using ELFA (Enzyme-Linked Floureshence Assay) technique, by VIDAS system (GCS KC Immunoassays, 2013). All the data recived from these samples, are collected and organized in excel and further statistical analysis of the data is done by SPPS 20 program for windows. Data analysis is based on the comparison of vitamin D levels between genders and different age groups, as well as on the comparison of vitamin D level between healthy individuals and those with parathyroid problems. According to the latest updates on reference levels for vitamin D, it is considered deficiency when the concentration is < 20 ng/ml; insufficiency when the value is between 20-29 ng/ml and sufficiency (normal level) when the concentration is from 30-100 ng/ml. As vitamin D is a fat soluble vitamin, high levels of it, > 100 ng/ml, can be a risk for vitamin D toxicity or hypervitaminosis D (although it is a very rare condition, caused by excess vitamin D).

Although VDT is rare, the health effects can be serious if it is not promptly identified. Many forms of exogenous (iatrogenic) and endogenous VDT exist. According to the recent studies, serum 25-hydroxyvitamin

D [25(OH)D] concentrations higher than 150 ng/ml (375 nmol/l) are the hallmark of VDT due to vitamin D overdosing (Marcinowska-Suchowierska, 2018). The received datas are tested and assesed about their distribution. From Kolmogorov-Smirnov test ($p < 0.05$), it turns out that our data does not have a normal distribution, so we will be limited mainly to non-parametric tests, such as Chi-square test, Fisher exact test, Mann-Whitney test dhe Kruskall-Wallis.

Results and Discussion

Albania, with a favorable geographical position in the Mediterranean basin, has very favorable climatic conditions for the use of solar radiation. As it is mentioned in many studies, sun exposure and solar radiation is the main source of vitamin D synthesis in our body through a cascade of reactions that begin in the skin. The aim of our study was to assess the actual status of vitamin D in a group of randomly chosen individuals and in a compared way with another group of randomly chosen ten years ago.A group of 288 adult individuals from 20 to 83 years old with a mean age 49.33 (±15.4) years old, was included in this study. 73.6% are females and 26.4% are males.

Table 1. Vitamin D levels in both groups included in the study

Year	Vitamin D (ng/ml)	
2009-2010	Mean (±SD)	21.49 (±7.6)
	Min.	4
	Max.	49
2019-2020	Mean (±SD)	26.07 (±10.06)
	Min.	8
	Max.	64

As can be seen from the box plot in figure 1 and the table above, which presents the vitamin D concentration, the average of vitamin D level, is higher in the individuals from the second group (26.07±10.06), in the period of time from 2019 to 2020 than the first group (21.49±7.6), from 2009 to 2010. The minimum and maximum values result to be slightly different between those groups. Although there are higher vitamin D levels in the second group, compared to the first group from ten years ago, 72% of our sample results to be in the levels of lack or insufficiency of vitamin D level. There can be different reasons to cause this, starting from daily diet variety, non-enough exposure to the sun (Greene-Finestone et al., 2011, Klingberg et al., 2015, Michalczyk et al. 2020), etc. This finding emphasizes the need for monitoring and observing the levels of vitamin D over the years as well as and the need for better awareness policy in our population.

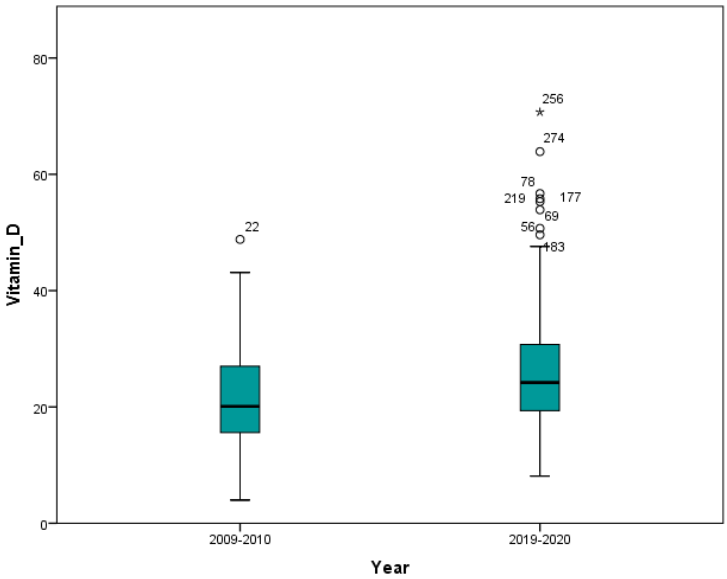

Figure 1. Comparison of vitamin D status between 2009-2010 group and 2019-2020 group

In addition to the graphical representation, which shows clearly the difference of vitamin D concentration between the two groups, from two different periods (ten years difference), we applied different statistical tests to

approve the difference. Based on the statistical test (Mann-Whitney, $p < 0.05$) it is shown that the distribution of the measurable variable, vitamin D, is not the same in both groups ($U = 7472$ and $p = 0.001$). The value of vitamin D is higher in persons belonging to the second group.

Table 2. Comparison of vitamin D status between males and females (in both groups)

Gender	Year	Vitamin D (Mean±SD) ng/ml
Females	2009-2010	20.89 (±6.8)
	2019-2020	25.01 (±9.4)
Males	2009-2010	22.49 (±8.3)
	2019-2020	31.16 (±11.39)

As can be seen from the box plot and mean values of vitamin D concentration, there is observed a higher level of vitamin D in males in both groups (2009-2010 and 2019-2020) comparing to females. The sample of the first group, from 2009 to 2010 consists of 62% females and 32% males. The mean of vitamin D level in males in the first group is 22.49 ng / ml (± 8.5) while in females 20.89 ng / ml (± 6.9). the sample of the second group, from 2019 to 2020 consists of 82.9% females and 17.1% males. The average vitamin D in men is 31.16 ng / ml (± 11.39) while in women is 25.01 ng / ml (± 9.4).

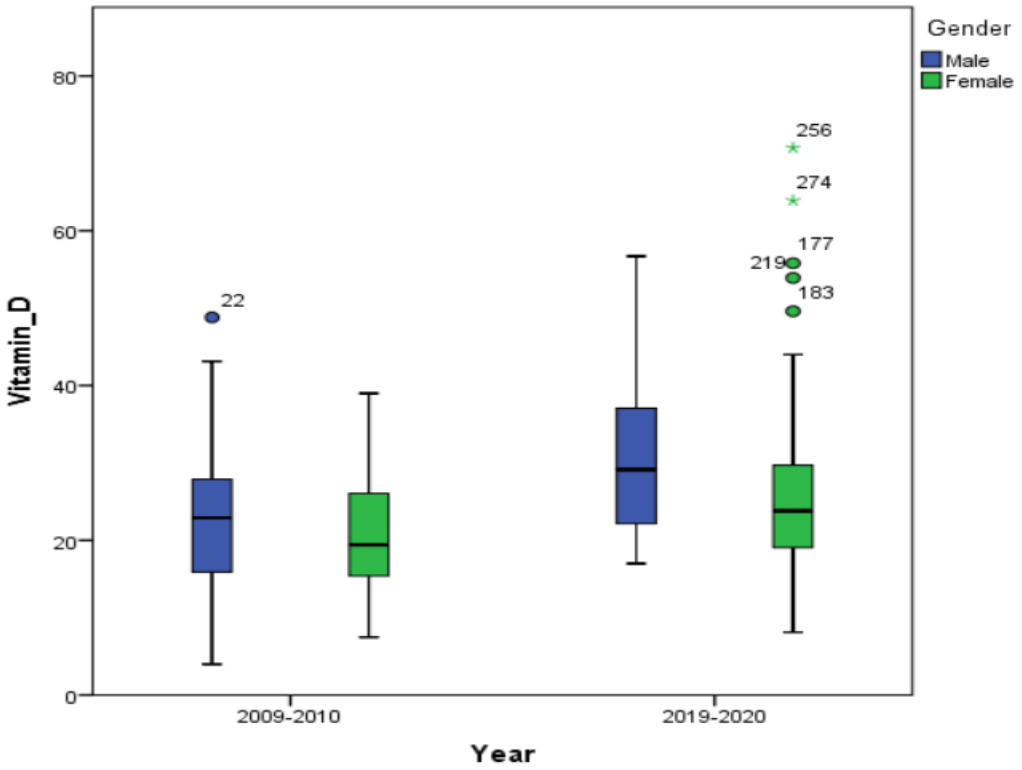

Figure 2. Comparison of vitamin D status between males and females in the two groups

During the period of time from 2009 to 2010 more than half of the women, about 53.8% had vitamin D deficiency, a significant part 37.5% have presented insufficiency while a very small part of the group, about 8.8% were considered healthy. The situation during the last years seems more positive related to vitamin D status in women. We found smaller percentage of women with vitamin D deficiency, about 32.1%, most of the women had vitamin D insufficiency, about 45%, and 22.9% of the women were healthy (a higher percentage of compared to the previous years). In men the situation is even better. We observed a decrease in the number of men with vitamin D deficiency, from 42.9% in 2009-2010 to 14.85 in recent years; we found out also a decrease in the number of men with vitamin D insufficiency from 42.9% to 33.3 %, which is followed by a significant increase in numbers of healthy individuals from 14.3% to 51.9%. In order to be sure, that there is a real improvement of the vitamin D status in our population, further studies on this direction are needed, including more people (bigger sample size).

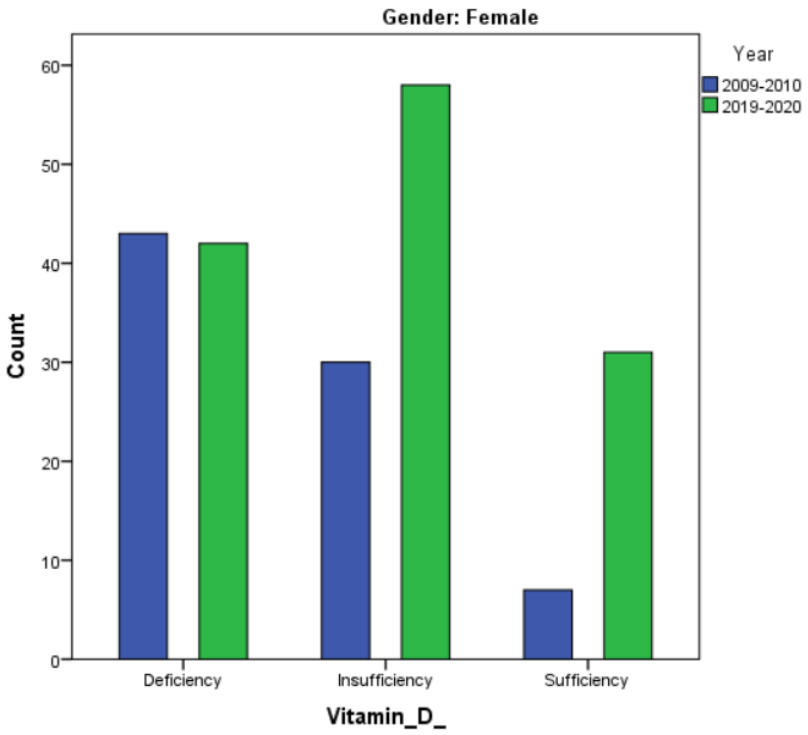

Figure 3. Comparison of vitamin D status in females during 2009-2010 and 2019-2020

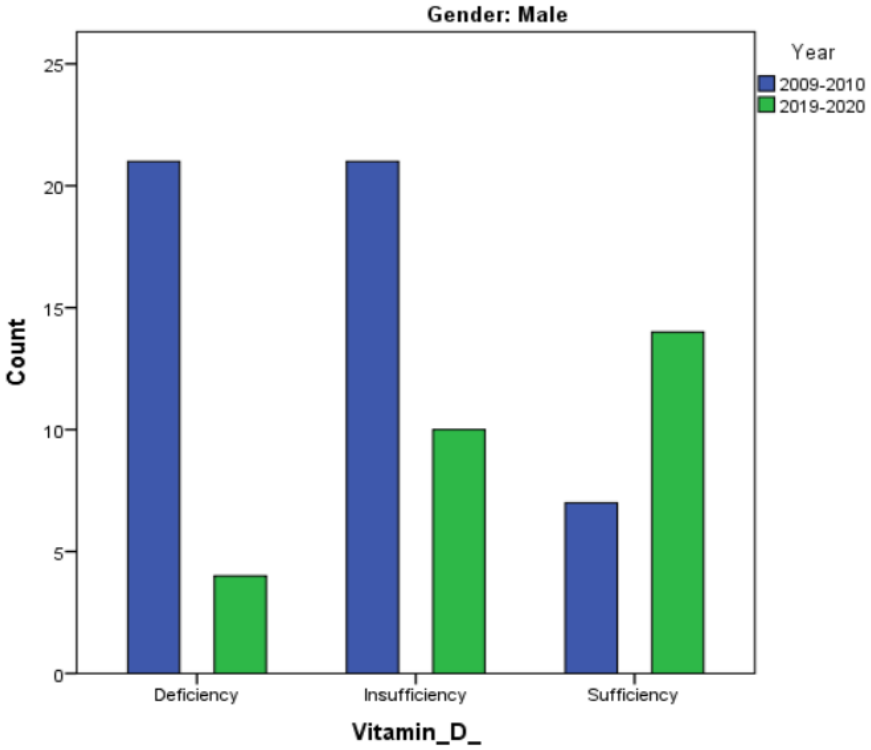

Figure 4. Comparison of vitamin D status in males during 2009-2010 and 2019-2020

In the graphs in figure 3 and 4, we have presented in a compared way vitamin D status, between males and females in the two groups (2009-2010 and 2019-2020) based on the reference level of vitamin D. In the figure 3 we can see the status of vitamin D in females and in figure 4 the status of vitamin D in males. From the data presented in figure 3, it is noticed that the concentration level of vitamin D, is higher in the females of the second group (2019-2020) when compared to the first group, ten years ago (2009-2010). The mean value of vitamin D in the first group is 20.89 (±6.9) ng/ml and the mean value of vitamin D in the second group is 25.01

(±9.4) ng/ml. As it seen from the mean value of the second group, in females after ten years there is a slightly increase in the level of active vitamin D.

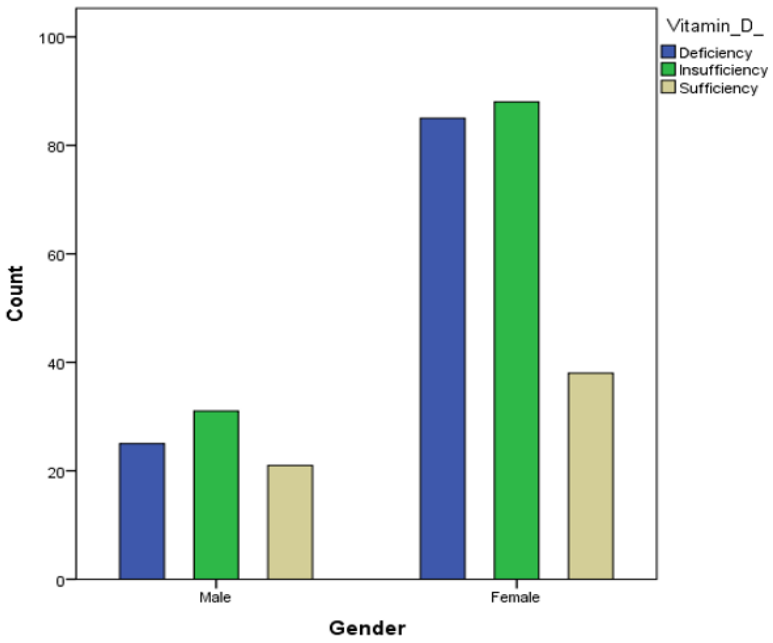

Figure 5. Relationship between gender and vitamin D

From data analysis it is noticed a significant relationship between gender and vitamin D (Fisher's exact test, p=0.017). Most of the cases with vitamin D deficiency are observed in females, which explains why women are more at risk and predisposed to be affected by diseases as a result of vitamin D deficiency. Recent studies show that in women there are more polymorphisms of the genes encoding for vitamin D receptors and proteins needed in the synthesis pathway of vitamin D, which lead to a series of mutations. It explains why women suffer more from vitamin D deficiency (Arosio et al., 2020). Findings from other authors in this field, reveal a cumulative effect of specific VDR gene polymorphisms that may regulate vitamin D concentrations explaining, in part, the paradox of vitamin D deficiency in sunny regions, with important implications for precision medicine (Divanoglou et al., 2021).

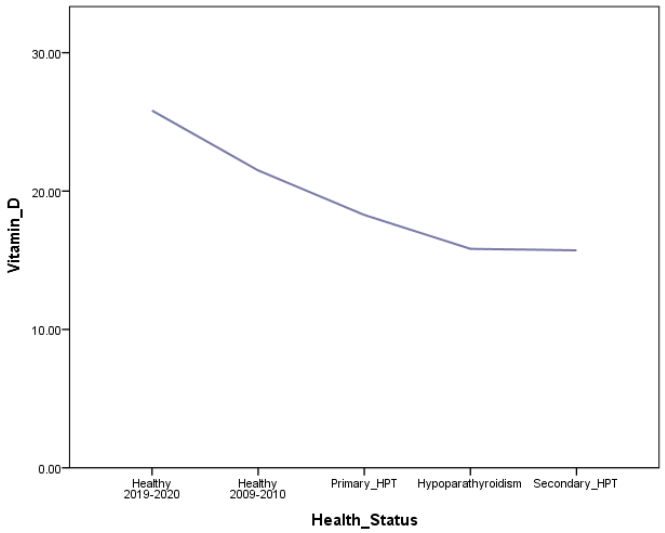

Figure 6. Comparison of Vitamin D in different health status groups

Parathyroid gland abnormalities/or linked diseases, as we can see from the graph affect the level of vitamin D in this group. From the data collected and the distribution of vitamin D, we can conclude that this parameter is statistically different between the 5 groups included in the study and presented in figure 6 (Kruskall Wallis test, P = 0.001). Vitamin D concentration in both healthy groups is higher than the other groups with parathyroid

problems. According to other studies findings, not all the individuals with vitamin D deficiency have high levels of PTH, a finding that agrees with the need for new criteria in the management of vitamin D deficiency and the importance of PTH testing (Malik et al., 2020).

Parathyroid gland problems affect the level of vitamin D concentration in the blood by reducing it, due to renal problems that occur in these individuals, causing a lack of maturation of vitamin D in the kidneys (final stage of hydroxylation). The situation is severe in secondary parathyroid problems as we can see from the graph and other studies in this field.

Deficiency in vitamin D causes secondary hyperparathyroidism, high bone turnover, bone loss, mineralization defects, and hip and other fractures (Lips, 2001). From diferent studies we see that vitamin D insufficiency can cause secondary hyperparathyroidism and chronic kidney diseases can lead also to vitamin D defficiency. On the other hand parathyroid hormone (PTH) can regulate the production of active vitamin D. Studies confirm also that the prevalence of vitamin D deficiency and insufficiency is greater across the stages of chronic kidney disease (CKD) than in the general population (Holick, 2007, Blair et al., 2008, LeClair et al., 2005). In CKD stages 3 and 4, up to two-thirds of patients are reported to have vitamin D insufficiency. In CKD stage 5, up to 97% of patients have vitamin D insufficiency (LeClair et al., 2005; Levin et al., 2007; Gonzalez et al., 2004). SHPT affects 40-80% of patients with stage 3 or 4 CKD and 95% of patients with Stage 5 CKD. CKD patients should be monitored for SHPT and effectively treated for underlying vitamin D deficiency in or-der to control SHPT and potentially reduce morbidity.

The evidences show that with the aging of the individual, the level of vitamin D in the circulation decreases. It is thought that the main factors that lead to such a thing is because with age the absorption of calcium in the intestine decreases, the renal production of 1.25 (OH) 2D decreases, the synthesis of vitamin D in the skin decreases and we also have a decrease of vitamin D substrates (Gallagher et al., 2013, Martins, et al., 2017). Further studies are needed in this point, due to some limitations on the sample size.

Conclusion

Most of the individuals tested, resulted to have vitamin deficiency or insufficiency (about 72%), although the situation of vitamin D status (in Tirana area) is better during 2019-2020 compared to ten years ago, from 2009-2010. This improvement of the situation can probably be due to the increased awareness about vitamin D importance to human health or it can be due to vitamin D supplements people were taking because of the warning against covid-19 and the importance of vitamin D in immune system boosting. There is a significant link between gender and vitamin D status, as it was seen that in most of the cases, women had vitamin D insufficiency. From this study we see how problems related to the parathyroid glands directly affect the status of vitamin D in the body. The worst conditions are observed in people suffering from hypoparathyroidism and dialysis patients.

Recommendations

Taking into consideration the fact that the levels of vitamin D remain low in our population and the insufficiency/deficiency is still high among people of different age-groups (despite the improvements), it is necessary to observe and assess continuously the vitamin D status in the Albanian population. Population screening is necessary in order to have a clearer view, so better directions and orientations can be given by the health professionals, parallel to the rise of the vitamin D deficiency awareness (health education and information sharing with the population, etc.).

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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