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DEFICIENCY OF VITAMIN D IN HIV INFECTED PATIENTS AND ITS EFFECT ON SOME OF THE BIOCHEMICAL PARAMETERS

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***Keywords**: HIV, 25(OH)D deficiency, insufficiency, nonclassical effects of Vitamin D*

Abstract
Today the HIV infection is a chronic disease with significantly longer duration of the life of the patients. Different factors which could affect the progress of the HIV infection and some comorbidities are being researched. Vitamin D (25(OH)D) is one of those factors, which interacts with a corresponding receptor (VDR), which is expressed by a large number of organs including brain, muscle, pancreas, colon, mammary glands and immune cells. The activation of those receptors is responsible for the so called „nonclassical" effects of Vitamin D. The aim of our study was to assess 25-hydroxyvitamin D (vitamin D) status in an HIV-infected adult population, to define factors associated with vitamin D deficiency and to assess some „nonclassical" effects of Vitamin D (association between 25-hydroxyvitamin D (vitamin D) status and some major biochemical parameters). The study includes 145 HIV – positive patients on treatment who are being monitored in the Department for acquired immune deficiency at Specialized Hospital for Infectious and Parasitic Diseases "Proff. Iv.Kirov"-Sofia. *The results* show that most of the tested patients are with deficiency or insufficiency of 25(OH)D. *In our survey we didn't found a statistically significant link between the* 25(OH)D serum levels and the season-factor. We also didn't found significant differences between the 25(OH)D serum levels according to gender and age of the tested HIV-positive patients. We found reliable dependence according to the performed treatment with the Efavirenz medicament only for women. *In patients with* 25(OH)D *deficiency we found significant reverse correlations between the* 25(OH)D serum levels and blood sugar values and the ALT. The decrease of the 25(OH)D serum level correlated with the increase of the blood glucose and ALT. *Positive correlation between the* 25(OH)D serum levels and the creatinine, we found only in the patients with normal 25(OH)D serum levels.

Introduction
Today the HIV infection has turned into a chronic disease with significantly longer duration of the life of the patient, as long as they maintain a suitable antiretroviral therapy. Different factors which could affect the immune system and the progress of the HIV infection are being researched. Vitamin D (25(OH)D) is one of those factors (3,4,8). In order to show its functions, 25(OH)D interacts with a corresponding receptor (VDR), which is expressed by a large number of organs including brain, muscle, pancreas, colon, mammary glands and immune cells (1,12). The activation of those receptors for 25(OH)D is responsible for the so called nonclassical effects of Vitamin D (5). Some researches show a link between the serum levels of 25(OH)D and some autoimmune diseases, Type 1 Diabetes Mellitus, cardiovascular diseases, cancer and infections (6,7,10,11,14,16).

Numerous international studies analyze the serum levels of 25(OH)D for HIV – infected patients, and the results show that those patients have deficiency and insufficiency more frequently than common population (2,3,4,8,9,13). The evaluation of the risk factors for the hypovitaminosis D for the HIV infection includes evaluation of the HIV-

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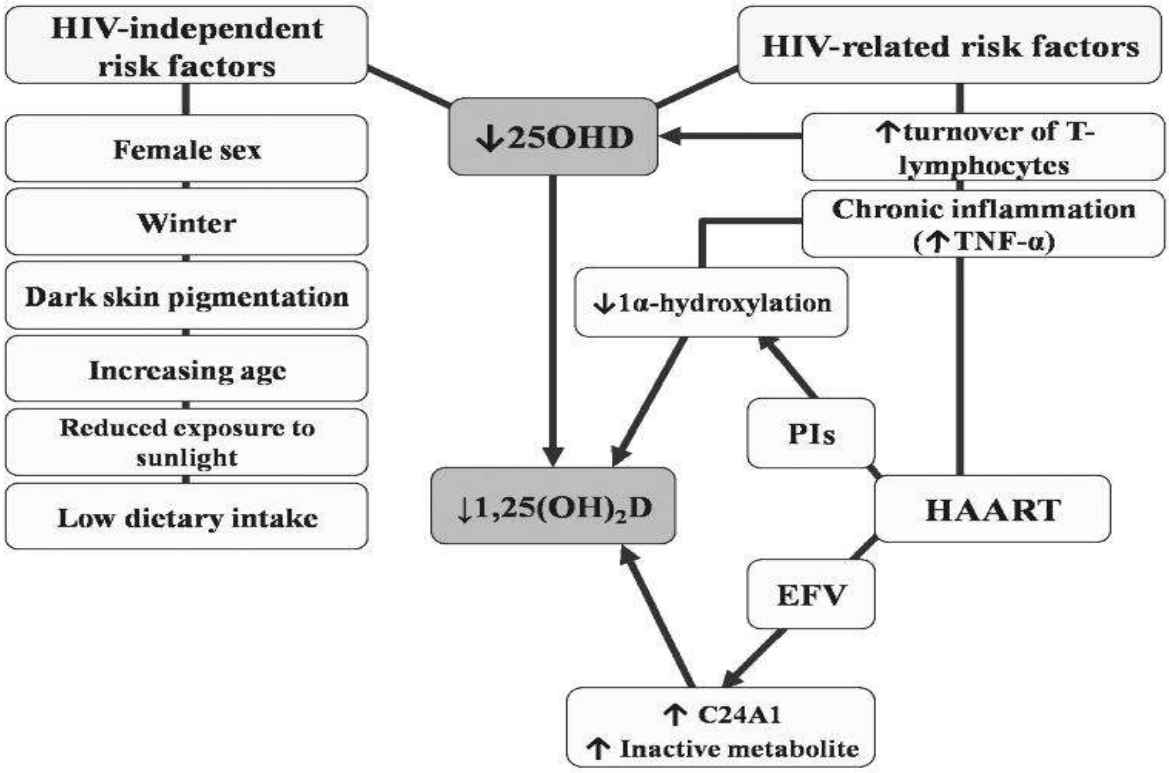
specific and HIV- independent risk factors. Some risk factors as the female sex, winter season, age, diet with a lower intake of vitamin D and a darker skin color are similar risk factors to those reported from the HIV-negative cohorts. Risk factors related to HIV-infection are increased turnover of the T- lymphocytes, chronic inflammation, increased TNF-α and the conducted antiretroviral therapy (3). The antiretroviral drugs which are proven to affect the metabolism of 25(OH)D are the protease inhibitors and the non-nucleoside inhibitor Efavirenz (EFV). The protease inhibitors inhibit 25(OH)D 1α- and 25α- hydroxylation in the hepatocytic and monocytic cell lines (8). The Efavirenz (EFV) medicament leads to reduction of the 25(OH)D catabolism, via induction of CYP24 and a decrease of the transcription from one 25- hydroxylase - CYP2R1 (13) (***Figure 1.)***

Figure 1. Risk factors for hypovitaminosis D when there's a HIV infection (M.Pinzone et all.).



The serum level impact of 25(OH)D upon the insulin secretion has been found out (6). 25(OH)D participates in the regulation of the insulin secretion and the insulin-mediated glucose transport. The results from numerous surveys shows low serum levels of 25(OH)D among the patients with type 2 diabetes, compared to patients without diabetes (10,11,14). Supplementation with Vitamin D leads to reduction of the insulin resistance with patients who have diabetes but are not HIV-infected. But there's still not enough data about patients with HIV.

A research conducted by Van den Bout surprisingly showed that the supplementation with cholecalciferol (2000 IU/ daily for 14 weeks, 1000 IU/ daily to 48 weeks) led to increased insulin resistance and elevated levels of fasting blood glucose, tested during the 24th week. There weren't any differences after the 48th week. The authors explain the results with the inhibitory effect of 25(OH)D upon the expression of PPAR-γ receptor. The authors assume that the effect of vitamin D on the insulin secretion and blood glucose level is dose and time-dependent (15).

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Objectives

The aim of our study was to assess 25-hydroxyvitamin D (vitamin D) status in an HIV-infected adult population, to define factors associated with vitamin D deficiency and to assess association between 25-hydroxyvitamin D (vitamin D) status and some major biochemical parameters.

Material and methods

The study includes 145 HIV – positive patients on treatment who are being monitored in the Department for acquired immune deficiency at Specialized Hospital for Infectious and Parasitic Diseases "Proff. Iv.Kirov"-Sofia.

The distribution of the patients according to their gender is shown on ***Figure 2.***

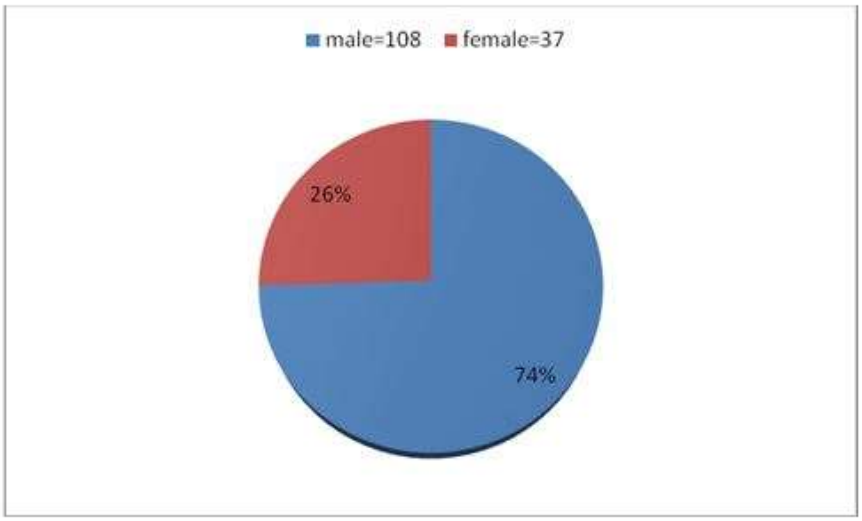

Figure 2. Percentage distribution by gender of the HIV-positive patients that are being monitored.

The average overall age of the tested subjects is 34.9 ± 9.3 years. The average age of the males is 34.8 ± 9.2 years and that of the females is 35.2 ± 9.5.

The patients are being monitored ambulatory; they did not have any indications for hospitalization. In the test group, bigger part of the patients is treated with the antiretroviral therapy, but there are also untreated (naive) patients. Part from the conducted researches are carried out routinely during the dispensary tracking of the HIV-positive – CBC, ALT, blood glucose, creatinnine, cholesterol and CD panel. A specialized test of the 25(OH)D serum level was conducted and the blood samples were collected in the months of November to March. The winter months were chosen intentionally in order to limit the impact of the sun light upon 25(OH)D serum level. The 25(OH)D (25OHDtotal) *serum level of all of the tested patients at first was determined by a highly sensitive and specific radioimmunoassay kit* (25OH VitaminDtotal-RIA-CT, DIAsource ImmunoAssays, Belgium). *According to the reference values of the method,* 25 (OH)D<10 ng/ml levels are defined as a deficiency and the serum levels between 10-30 ng/ml are defined as insufficiency. It's sufficient if the level of 25 (OH)D is from 30 to 150 ng/ml.

After testing 25(OH)D for a second time, in the second stage of the study 60 patients from the group had enzyme immunoassay with highly fluorescent measurement ELFA. The test VIDAS® 25 OH Vitamin D Total was used. The test is quantitative and it uses the technology of the enzyme fluorescence assay research ELFA (Enzyme Linked

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Fluorescent Assay). According to the reference values of this method the 25(OH)D<20 ng/ml serum levels are considered as deficit and the serum levels between 20-30 ng/ml are defined as scarcity. It's sufficient if the level of 25(OH)D is 30 to 100 ng/ml.

Results and discussion

From all of the monitored patients only in 15% of the tested (n=22) we found normal 25 (OH)D serum levels. Accordingly, for the other 85 % (n=123) we discovered 25 (OH)D < 30 ng/ml serum levels. From them 12 % (n=17) the 25 (OH)D serum level is < 10 ng/ml, which defines as deficiency of Vitamin D. The largest group is that of patients with 25 (OH)D level between 10-30 ng/ml - 73 % (n=105). *Those serum levels are defined as deficiency of vitamin D.* **(Figure 3.)**

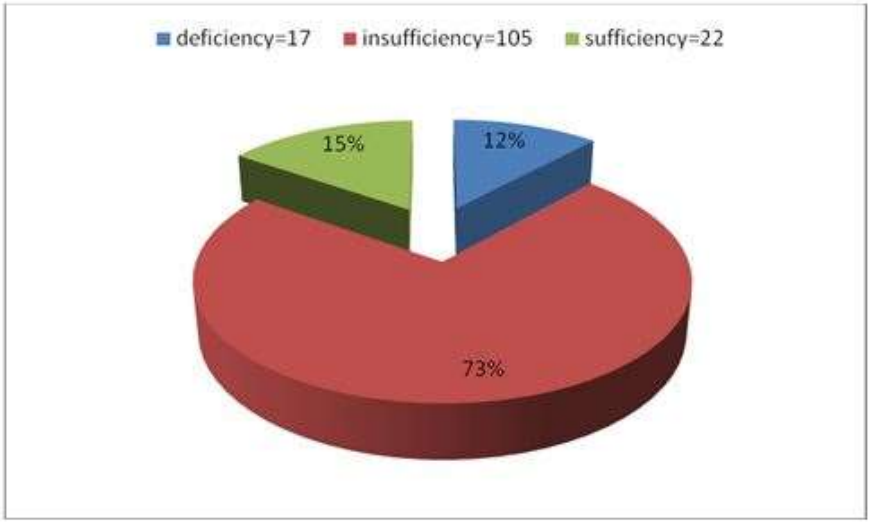

Figure 3. Distribution of patients according to their 25(OH)D serum level

We didn't discovered significant difference in the 25 (OH)D average values between men and women (Mann-Whitney U test, Z = 0.18595, p = 0.8524832). From the tested women 83% (n=31) have values of 25 (OH)D below 30 ng/ml. The tested men 83, 3 % (n= 90) had deficiency or insufficiency of 25 (OH)D.

There were no significant differences between the average values of 25(OH)D of the male and female patients and in the individual groups defined as deficiency, insufficiency and sufficient levels of vitamin D. We also didn't found significant differences between the 25(OH)D serum levels according to age of the tested HIV-positive patients

There were no significant differences in the average values of the 25(OH)D serum levels when dividing the patients according to intake and their type antiretroviral therapy in 4 groups (naïve patients, treated with protease inhibitors, treated with Efavirenz and treated with Nevirapine).

After separating the patients by gender, there was a statistically significant association with the 25(OH)D level and the conducted treatment only in women. The untreated women had average values of 25 (OH)D higher than that of the women treated with EFV(MSE = 188.59, df = 33, p = 0.026746). Furthermore, the average value of 25 (OH)D in the untreated women was indeed higher than that of the untreated males(p = 0.0257432).

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After a re-examination of 25(OH)D - 60 patients from the group, although the blood samples were collected between the summer and autumn months, we once again found that a big part from the patients have deficiency or insufficiency. From the tested patients 81,6 % (n=49) were with 25(OH)D values lower than the normal, and for 41,6 % (n=25) of them we discovered deficiency, and for 40% (n=24) of them we discovered insufficiency. Only 18,4 % (n=11) of the patients had normal 25(OH)D values (fig.3)

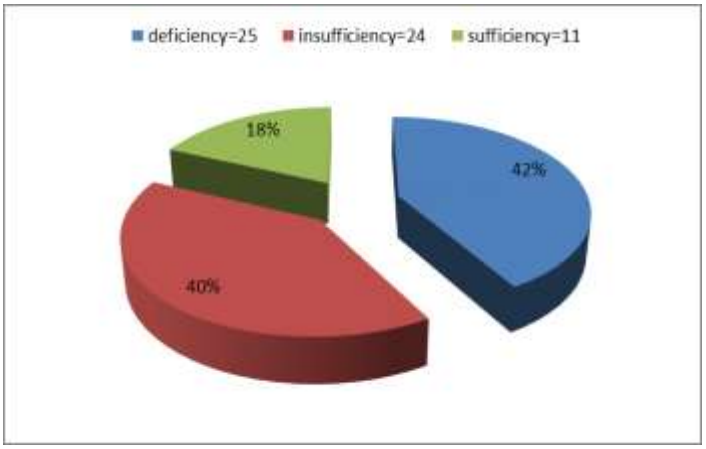

Fig.3 Distribution of patients according to their 25(OH)D serum level at the second stage of survey

By using correlation analysis we evaluated possible correlations between the 25(OH)D serum levels and the *biochemical parameters - blood glucose, cholesterol, creatinine, and ALT.*

In patients with 25(OH) D *deficiency we found significant reverse correlations between the* 25(OH)D serum levels and blood sugar values (r=-0.47, p=0.038655) and the ALT (r=-0.58, p=0.01356). The decrease of the 25(OH)D serum level correlated with the increase of the blood glucose and ALT (*Table 1*).

Table 1. Correlation between 25(OH)D levels and biochemical parameters in all patients

25(OH)D	glucose	Total cholesterol	ALT	creatinine	p
Deficiency <10 ng/ml (n=13)	**-0.47**	**0.309**	**-0.58**	**-0.242**	**p=0.038655 p=0.01356**
Insufficiency 10-30 ng/ml (n = 108)	**-0.071**	**-0.067**	**0.0071**	**0.016**	
Sufficiency >30 ng/ml (n=24)	**0.36**	**-0.025**	**0.09**	**0.35**	**P<0.05**

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After dividing the patients according to the sex, we found the same correlation in male group (Table 2).

For women with normal 25(OH)D levels we discovered statistically significant positive correlations between 25(OH)D serum levels and the blood glucose (r=0.8, p<0.05). *We found positive correlation between the* 25(OH)D serum levels and the creatinine in the group with normal 25(OH)D (r=0.75, p<0.05) (Table 3).

Table 2. Correlation between 25(OH)D levels and biochemical parameters in the tested men

25(OH)D	glu	chol	ALT	creat	p
Deficiency <10 ng/ml (n=10)	-0.38	0.325	-0.58	-0.177	p<0.05
Insufficiency 10-30 ng/ml (n=80)	-0.011	-0.068	0.123	-0.076	
Sufficiency >30 ng/ml (n=18)	0.244	0.15	0.073	0.438	p<0.05

Table 3. Correlation between 25(OH)D levels and biochemical parameters in the tested women

25(OH)D	glucose	cholesterol	ALT	creatinine	p
Deficiency <10 ng/ml (n=3)	-0.12	0.23	- 0.0964	0.14	
Insufficiency 10-30 ng/ml (n = 28)	-0.189	-0.18	-0.191	0.25	
Sufficiency >30 ng/ml (n=6)	0.8	-0.16	-0.259	0.775	p<0.05

Summarizing these results we can say that our research confirms the data of other researches, that the effect of 25(OH)D upon the blood glucose is dose-dependent. When 25(OH)D (< 10 ng/ml) is in deficiency, the decrease of 25(OH)D serum levels correlate with an increase of the blood glucose. With the normal 25(OH)D levels, the increase of the serum level of 25(OH)D correlates with an increase in the blood glucose.

For the established correlation between the 25(OH)D deficiency and the increased ALT, the reasons are probably complex. There's the influence of the antiretroviral therapy upon the ALT results (treatment with Nevirapine), there's also the impact of 25(OH)D upon the glucose and lipid metabolism and respectively the deposition of fat in the liver.

A dose-dependent effect could also discussed for the established significant positive correlation with the creatinine level of the patients with normal serum 25(OH)D levels. Positive correlation between the 25(OH)D serum levels and the creatinine, we found only in the patients with normal 25(OH)D serum levels, meanwhile the patients with 25 (OH)D deficiency and insufficiency we didn't discover significant correlations with the serum creatinine values.

Conclusion

HIV – infected patients have deficiency and insufficiency more frequently than common population. *In our survey we didn't found a statistically significant link between the* 25(OH)D serum levels and some HIV- Independent factors - the season-factor, gender and age. We found reliable dependence according to the performed treatment with the one antriretroviral drug - Efavirenz. About tne" nonclassical" efects of vitamin D we found that the effect of 25(OH)D upon the blood glucose and creatinine is dose-dependent. In cases of deficiency, the decrease of 25(OH)D serum levels correlate with an increase of the blood glucose. With the normal 25(OH)D levels, the increase of the serum level of 25(OH)D correlates with an increase in the blood glucose and creatinine. We *established* correlation between the 25(OH)D deficiency and the increased ALT, but the reasons are probably complex – the influence of the antiretroviral therapy and the impact of 25(OH)D upon the glucose and lipid metabolism and respectively the deposition of fat in the liver.

In summary, our data suggests that suplementation with vitamin D in cases with deficiency or insufficciensy of 25(OH)D in HIV – infected patients is important not only for classical effect of vitamin D, but and for other biochemical parametres and respectively comorbidities.

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