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Vitamin D Associated Peculiarities in Women with Mild Covid-19 and Effect of Calcifediol on the Level of Vitamin D and Possibly, on Disease Outcome - Prospective Pilot Study

Grigoryan Karen[1], Mikaberidze Khatia[1], Ratiani Levan[1], Goginashvili Nino[1], Pachkoria Elene[1], Gabunia Luiza[2], Sanikidze Tamar[4], Pkhaladze Maia[1], Metreveli Levan[1], Ghambashidze Ketevan[3]

Tbilisi State Medical University (TSMU) First University Clinic[1];
Department of Medical Pharmacology and Pharmacotherapy[2];
Department of Pathophysiology[3];
Department of Physics, biophysics, biomechanics and information technology[4].

Abstract – **Presented study provides information about prospective pilot study concerning the vitamin D in women with mild COVID-19 (SARSCoV2) and effect of Calcifediol on the vitamin D level and possibly, on disease outcome.**
The study involved 30 women aged 48 to 72 years who were treated for a mild form of new coronavirus infection between 1.09.2020 - 1.12.2020 at the First University Clinic of TSMU.
In all patients, the new type SARSCoV2 was confirmed by polymerase chain reaction performed with reagents-Xpert Xpress SARS-CoV-2 (Cepheid, USA) on GeneXpert (USA) analyzer (closed system). D-dimer, ferritin, C-reactive protein and lactate dehydrogenase were detected upon the hospital admission. Vitamin D was defined using Roche reagents (electrochemiluminescence method; Roche HITACHICobase-411 device- Switzerland).
On the 1st day of hospital admission, all patients received 0.266 mg (1 capsule) of calcifediol (16000 UI) in the form of the drug Hydroferol (FAES FARMA, S.A., Spain). Vitamin D and -reactive protein was defined repeatedly on the 5th day of treatment.
Results: Studies have shown that on the 5th day after hydroferol administration the vitamin D level in patients with COVID-19 was significantly increased [16.8 (12.2–24.2) vs. 56.4 (52.3–62.2), p<0.0001]. Negative relationship was found between the onset of symptoms and baseline vitamin D levels (F = 5.9223, p <0.026). The negative relationship was found between the repeatedly defined levels of vitamin D and C-reactive protein (F=12.82, p<0.009) also, pointing on the positive treatment effect of vitamin D on the course of mild form of Covid-19. None of the patients required continued treatment in the intensive care unit and were discharged home with satisfactory state.
Conclusion: Vitamin D supposedly plays the important role in pathogenesis and outcome of SARSCoV2 virus infected patients. Additional large-scale randomized placebo-controlled studies on the role of vitamin D in COVID-19 pathogenesis are needed.

Keywords – Vitamin D, Covid-19, Calcifediol, Women.

I. INTRODUCTION

Vitamin D deficiency is a worldwide problem with health consequences that cannot be under estimated. It is common not only for population residing in northern countries due to the lack of sunlight. Nowadays, it is recognized as a pandemic [1], [2].

Vitamin D is a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and many other biological effects. The major natural source of the vitamin D (Cholecalciferol) is the synthesis of

Corresponding Author: Ghambashidze Ketevan

cholecalciferol in the lower layers of skin epidermis through a chemical reaction that is dependent on sun exposure (specifically UVB radiation). It is converted in the liver to calcifediol (25-OH-hydroxyvitamin D), which is then converted in the kidneys owing to fibroblast growth factor to the active form of vitamin D - calcitriol (1,25-dihydroxyvitamin D3) [3].

Vitamin D is important for not only the growing skeleton, but also plays an important role in reducing risk of many diseases. Although, one of its actions is to increase the uptake of calcium by the intestines, observational studies have shown an indirect relationship between its deficiency and the development of autoimmune processes, cardiovascular disease, diabetes mellitus and malignant tumors [4], [5], [6], [7], [8], [9]. Several meta-analyzes provide information about the positive effects of vitamin D on the prevention of acute respiratory tract infections [10], [11], [12], [13]. Accordingly, arises question, whether there is an etiopathogenic relationship between vitamin D and the disease COVID-19 (Corona Virus Disease 2019) caused by the new type SARSCoV2 (Severe acute Respiratory Syndrome-Coronavirus 2). We suppose that administration of vitamin D owing to its unique properties could contribute the mild form of disease course and reduce lethal outcome in patients with COVID-19.

Studies are ongoing in this direction [14], [15], [16], [17] and one of the prospective pilot studies were conducted in Georgia as well, in particular, on the basis of one of the largest "Covid hospitals" in Georgia - the First University Clinic of TSMU.

II. Material and Methods

The total of 30 women aged 48 to 72 years who were treated for a new coronavirus infection between 1.09.2020 - 1.12.2020 at the First University Clinic of TSMU were involved in presented study. Algorithm for inclusion of patients in the study is presented on Figure 1.

In all patients, the new type SARSCoV2 was confirmed by polymerase chain reaction performed with reagents-Xpert Xpress SARS-CoV-2 (Cepheid, USA) on GeneXpert (USA) analyzer (closed system).

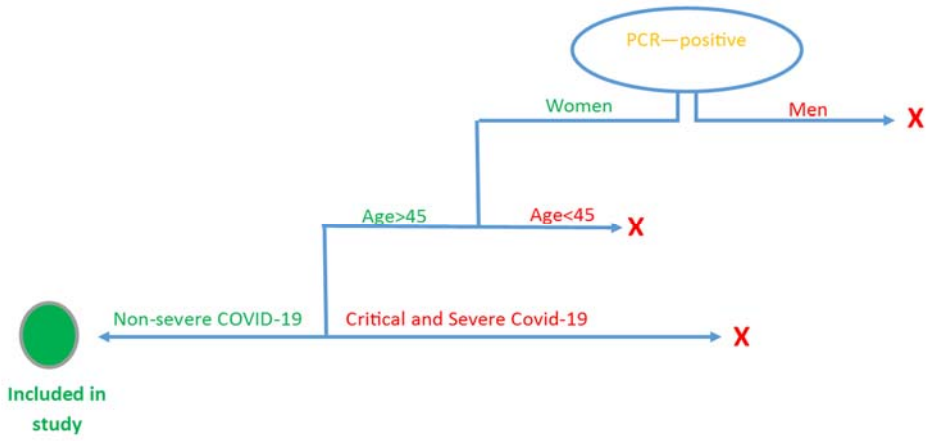

Fig. 1. Algorithm for inclusion of patients in the study.

Red X – patients, not included in study (exclusion criterion);
Green circle – patients, included in study (inclusion criterion)

Prior to enrolling in the study, patients signed an informed consent form (adopted in 1964 and subsequently amended in 2013 in accordance with the Declaration of Helsinki) that was approved by the Ethics Committee of Tbilisi State Medical University.

Out of 30 women, 24 (80%) persons underwent chest computed tomography (Toshiba-s Aquilion 16-layer tomography, Japan) upon admission to the hospital, and the remaining 6 (20%) underwent chest radiography (Schimadzu's X-ray device; RAD speed fit Plus, Japan).

Lung damage (LD) index was calculated by semi-quantitative method analysis, where the maximal LD score was equal to "24" and minimal – "0". D-dimer, ferritin, C-reactive protein and lactate dehydrogenase were detected upon the hospital admission.

Vitamin D was determined in the fasting patients' blood on the 2nd day of hospitalization with the use of Roche reagents (electrochemiluminescence method; Roche HITACHICobase-411 device- Switzerland). Reference intervals of the obtained laboratory parameters are presented on Table 1.

Tab. 1 Measured laboratory parameters reference intervals.

Parameters	Reference intervals
D-dimer	ng/ml < 243
Lactate dehydrogenase	U/L < 227
Ferritin	ng/ml 13– 150
C- reactive protein	mg/l < 5
Vitamin D	ng/ml > 24

After determination of vitamin D levels all patients received 0.266 mg (1 capsule) of Calcifediol (16000 UI) in the form of the drug Hydroferol (FAES FARMA, S.A., Spain). Vitamin D and C-reactive protein levels were repeatedly determined on the 5th day after drug administration. All patients were subjected to standard therapy (400 mg of hydroxychlorine - "Plaquenil" Sanofi-Synthelabo, Ltd., UK; and 500 mg of azithromycin - GMP, Georgia).

Qualitative data are represented by percentages, while quantitative results are represented by median and interquartile range [M (IQR)]. The comparison of Vitamin D level before and after Hydroferol administration was made by the Wilcoxon test.

Regression analysis was used to determine the relationship between vitamin D and various parameters. The p value<0.05 was considered significant. All p was calculated as two-sided. Statistical analysis was performed by Medcalc Statistical Program, version 18.2.1 (MedCalc Software Ltd, Belgium).

III. Results

Patients lab. test and age data are presented on Table 2. Studies have shown that patients with COVID-19 had a mild form of disease (C-reactive protein was slightly increased, lung damage index score was <50%, D-dimer and lactate dehydrogenase were within the reference range).

Tab. 2 Lab. test and age data of patients with COVID-19.

Parameters	M (IQR)
Age (years)	62 (54–64)
Number of days from the onset of symptoms to the Calcifediol administration	6 (5–7)
The sum of points of **LD** before Calcifediol administration	5 (1–7)
Leukocytes (10^9 / L) before Calcifediol administration	4.7 (4.1 –5.79)
Eosinophils (%) before Calcifediol administration	0.8 (0.3–1.0)
Neutrophils (%) before Calcifediol administration	58.8 (47.9–62.5)

Lymphocytes (%) before Calcifediol administration	31.8	(25 –43.3)
C-reactive protein (mg/l) **before** Calcifediol administration	8.1	(5.0–26.5)
C-reactive protein (mg/l) **after** Calcifediol administration	19.5	(9.5 –27.5)
D-dimer (ng/ml) before Calcifediol administration	92	(70–206)
Lactate dehydrogenase (U/L) before Calcifediol administration	237.9	(205.0– 295.0)
Ferritin (ng/ml) before Calcifediol administration	156	(60.1 –199.3)

On the 5th day of treatment with Calcifediol the blood vitamin D level in all patients was significantly increased and exceeded the lower limit of the reference interval (24) (Fig. 2, tab. 2), indicating the treatment efficacy of Calcifediol.

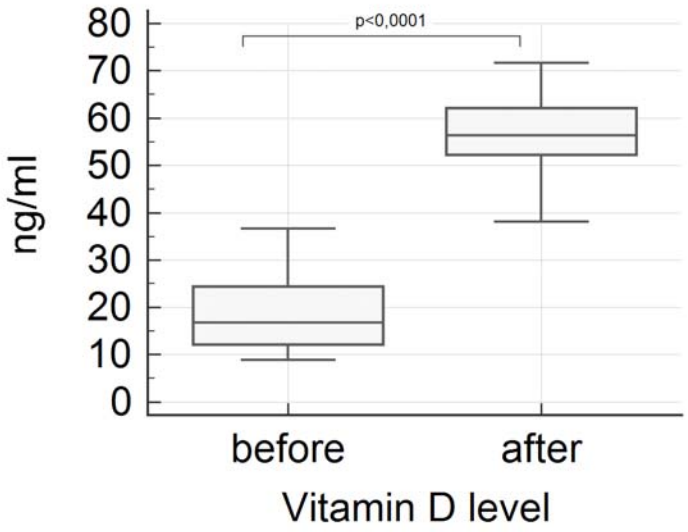

Fig. 2 Vitamin D level before and after treatment with Calcifediol.

In our carried out pilot study, we tried to establish association between vitamin D and some parameters. It was found that with increasing age, the level of vitamin D in the blood decreases significantly ($F = 20,888$, $p < 0.001$) (Figure 3).

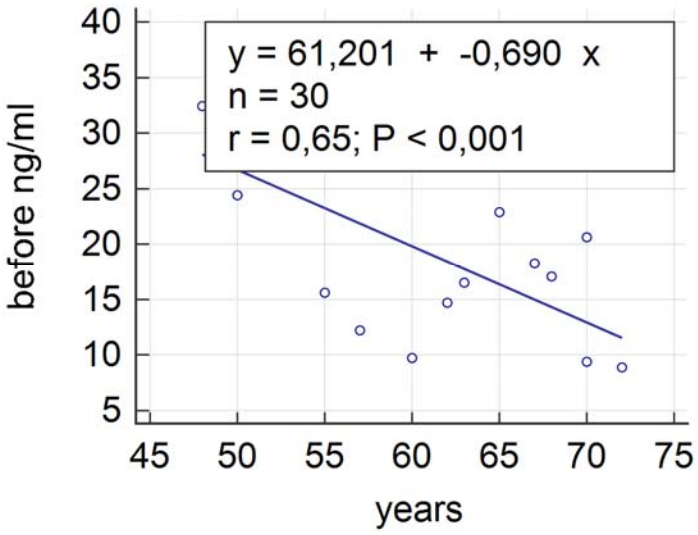

Fig. 3 Dispersion diagram between age and vitamin D.

Correlation was found between the onset of symptoms and the period before taking the drug and vitamin D level (Figure 4) as well.

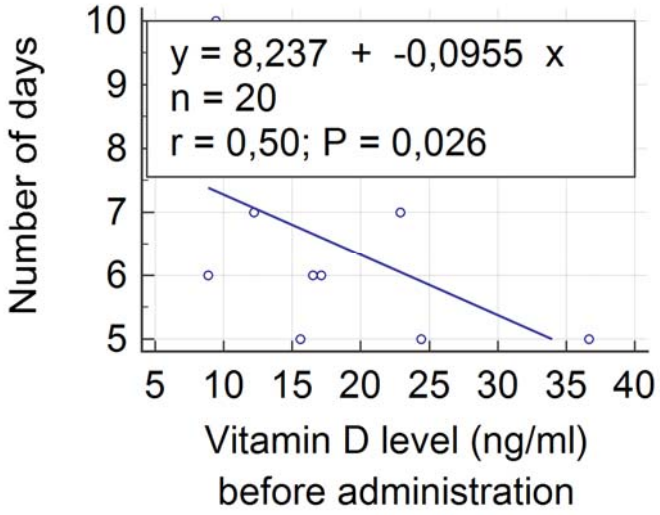

Fig. 4 Dispersion diagram between vitamin D and the period since the onset of symptoms.

Fig. 4 clearly shows that the longer the time elapsed since the onset of symptoms, the lower the level of vitamin D ($F = 5.9223$, $p < 0.026$) in the blood, which indirectly indicates the involvement and importance of vitamin D in the disease development.

Calcifediol increased vitamin D levels in the blood, which was also associated with re-defined C-reactive protein (the higher the level of vitamin D in the blood, the lower the rate of repeatedly defined C-reactive protein) ($F = 12.82$, $p < 0.009$).

IV. Discussion

The obtained results supposedly indirectly indicate the role of vitamin D in pathogenesis of SARSCoV 2 virus in women. There are studies in the literature where the positive effect of vitamin D on COVID-19 outcome has also been revealed. In a randomized study (COVIDIOL) carried out in Spain, investigators compared two groups of COVID-19 patients in which one group received 0.532 mg of cholecalciferol on the day of admission, followed by 0.266 mg of cholecalciferol on the 3rd and 7th day. While, the 2nd group patients were not treated with vitamin D supplements. Studies have shown that only 1 (2%) in the cholecalciferol treated

group patients required continued treatment in the intensive care unit, while in the control group - 13 (50%). The odds ratio for continuing treatment in the intensive care unit is 0.02 (95% confidence interval 0.002–0.17) [16].

The second study conducted in France has shown the sharp vitamin D deficiency in residents of nursing home. The study group I elderly with COVID-19, 1 month before or during Covid-19 illness received cholecalciferol. The control group II residents were not subjected to vitamin D therapy. The study has shown that survival percent in the group I patients treated with vitamin D was 82,5% (47). They required no continued treatment in the intensive care unit and were discharged home with satisfactory state. While in the control, group II patients survival percent was only 44.4% (4) [18], [19].

In our carried out pilot study none of the patients required continued treatment in the intensive care unit and they were discharged home with satisfactory state.

The disadvantage of our study is the small number of patients, which is also characteristic for Covidiol and the French quasi-experimental studies [16], [18]. The absence of randomization and control group is an essential disadvantage of our study also. However, the results obtained indirectly highlight the involvement of vitamin D in the pathogenesis of COVID-19.

At the same time, it should be mentioned that some relevant papers have not confirmed the etiological relationship between vitamin D and COVID-19. A prospective placebo-controlled study published in the January issue of the journal Lancet Diabetes & Endocrinology did not confirm the positive treatment effect of vitamin D on the prevention of respiratory infections in persons with vitamin D deficiency [20]. Furthermore, the journal Annals of Internal Medicine presents the results of a study that suggests that vitamin D 1000 UI overdose, on the contrary, may be harmful to patients and increase the risk of fractures, especially in the elderly [21].

The UK National Institute of Health in its guideline “Rapid Guideline”, describing the relevant literature reviews, notes that at present there is insufficient evidence for the preventive properties of vitamin D in the development of COVID-19 however, in the same literature review confirms the association between the vitamin D and course of COVID-19, but it is not an etiopathogenetical relationship. The literature review provides information about possible influence of inflammatory processes on the level of vitamin D [22], [23].

The German Association of Endocrinologists (Deutsche Gesellschaft für Endokrinologie) recommends a long stay in the fresh air. If it is impossible, the German experts recommend vitamin D supplement with the daily dose of 800-1000 UI for the prevention of vitamin D deficiency [24]. At the same time, experts do not rule out the positive effect of vitamin D in prevention of the COVID-19 development, that requires large-scale randomized placebo-controlled studies.

We also plan to conduct a randomized prospective study in the future involving 150 new patients with coronavirus infection (both women and men). As soon as the relevant results of the study are obtained, they will be published.

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