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Research Article

High Doses of Vitamin D3 In a Sub-Group of Covid-19 Patients Who Participated in Cared Clinical Study

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Abstract

Background: Previously, we evaluated angiotensin levels and the cytokine profile in patients with mild COVID-19 and treated with high doses of vitamin D3 (ClinicalTrials.gov NCT04411446). Unfortunately, this treatment did not improve the protective components.

Objectives: To perform a sub-study excluding old and obese patients as possible confounders.

Methods: From the initial 16 patients, we excluded those with BMI greater than 40 and those older than 70 and were randomized to a single oral dose of 500,000 IU vitamin D3 (n=7) or placebo (n=4). Ang II and Ang-(1-7) levels were determined by radioimmunoassay and interleukins (ILs) 1, 6, 8, and 10 and TNF-α by ELISA before and after treatment. Parallel, serum 25-OH vitamin D concentrations were measured by a chemiluminescence immunoassay.

Results: We verified a trend to the highest reduction in pro-inflammatory ILs and protective markers of Ang-(1-7) and Ang-(1-7)/Ang II ratio in the vitamin D3 treated group.

Conclusions: High-dose vitamin D3 supplementation in mild to moderate COVID-19-positive patients with plasma vitamin D levels within the normal range -even eliminating obesity and ageing- did not cause significant changes in clinical aspects.

Keywords: COVID-19; SARS-CoV-2; Vitamin D3; Angiotensin II; Angiotensin 1-7; Interleukins; Obesity; Aging

Introduction

It has been well-described that Angiotensin-converting enzyme 2 (ACE2) transforms Angiotensin II (Ang-II) into Ang-(1-7), an anti-inflammatory peptide. This enzyme may act dually, as it can mediate protective effects and work as a receptor for the SARS-CoV-2 virus,

causing the subsequent cytokine storm and the severe acute respiratory syndrome already known. This phenomenon is due to the sequestering of ACE2 by the virus, causes a loss in its catalytic effect, provoking an imbalance in the renin-angiotensin system (RAS) pro-inflammatory (ACE/Ang-II) and anti-inflammatory (ACE2/Ang-(1-7)) axes. Regarding this, there is evidence that vitamin D may down-regulate the RAS pro-inflammatory axis and up-regulate the anti-inflammatory one [1,2]. In this context, CARED-TRIAL (ClinicalTrials.gov NCT04411446) hypothesized that vitamin D could be a useful active compound in COVID-19 therapy. CARED-TRIAL used a cohort of 218 adult patients, of which only 16 agreed to participate in a pathophysiological sub-study. The latter were randomly treated with a single oral dose of 500,000 IU of vitamin D3 (n=10) or placebo (n=6). This sub-study aimed to assess plasma levels of Ang II, Ang-(1-7) and cytokines in individuals hospitalized for moderate COVID-19 and treated with vitamin D3 at high doses and to establish whether there is a correlation between vitamin D status and COVID-19 severity and prognosis. For this purpose, both before and after treatment, radioimmunoassay and ELISA were used to determine plasma levels of Ang II/Ang-(1-7) and interleukins (IL-1, 6, 8, 10 and TNF-α), respectively [3,4]. Unfortunately, vitamin D3 treatment did not improve the protective components. In this sense, we proposed to perform a new sub-study excluding old and obese patients as possible confounders.

The Ethics Committee of the Hospital El Cruce (Comité de Ética en Investigación Hospital de Alta Complejidad El Cruce) approved the study on 23 June 2020 (reference 36/2020). The local ethics committees of the participating institutions approved the study protocol before the start of the trial in each site. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association and with the principles of the Helsinki Declaration.

Patients enrolled in this new sub-study came from CARED-TRIAL conducted in hospitalized patients with a SARS-CoV-2 confirmed infection who meet the criteria to enroll in CARED to improve the outcomes of patients with COVID-19. In detail, it was a multicenter, randomized, double-blind, sequential, placebo-controlled trial, described previously [4].

From the initial 16 patients, we excluded those with body mass index (BMI) greater than 40 and those older than 70 and were randomized to a single oral dose of 500,000 IU vitamin D3 (n=7) or placebo (n=4). Ang II and Ang-(1-7) levels were determined by radioimmunoassay and interleukins (ILs) 1, 6, 8, and 10 and TNF-α by ELISA before and after treatment. Parallel, serum 25-OH vitamin D concentrations were measured by a chemiluminescence immunoassay. The data were analyzed by a statistician using SPSS Statistics 19 software. Kolmogorov–Smirnov test was applied to verify normal distribution, and Kruskal–Wallis's test was utilized to investigate differences. Statistical analysis was performed on each group (baseline vs after placebo treatment and baseline vs after vitamin D3 treatment) with paired t-test. At the same time, the differences between groups after treatments were analyzed with an unpaired t-test. Baseline and achieved concentrations were compared for each laboratory variable using paired t-tests. A $p<0.05$ was considered statistically significant.

Concerning the plasmatic vitamin D status of this sub-study and as expected, vitamin D3 supplementation significantly increased serum 25-OH vitamin D (baseline 37.40 ± 4.92 vs vitamin D3 treated 106.7 ± 10.66 ng/mL) In comparison, the placebo group did not change baseline levels (baseline 29.83 ± 3.43 vs placebo-treated 30.50 ± 2.12 ng/mL). Both differences in the vitamin D3 treated patient's vs baseline and with placebo-treated were significant, $p<0.001$.

The results exhibited a trend to a higher reduction in pro-inflammatory ILs (Il-6, Il-8, IL-1B, and TNF-α) in the vitamin D3 treated group (Il-6: baseline 24.3 ± 8.3 pg/mL vs vitamin D3 treated 13.9 ± 5.4 pg/mL, Il-8: baseline 18.6 ± 3.1 pg/mL vs vitamin D3 treated 11.6 ± 2.8 pg/mL, Il-1B: baseline 7.6 ± 0,8 pg/mL vs vitamin D3 treated 5.2 ± 0.5 pg/mL, TNF-α: baseline 12.4 ± 2.1 pg/mL vs vitamin D3 treated 7.6 ± 1.8 pg/mL) than in the placebo group (Il-6: baseline 24.3 ± 8.3 pg/mL vs placebo-treated 18.6 ± 9.1 pg/mL, Il-8: baseline 18.6 ± 3.1 pg/mL vs placebo-treated 14.7 ± 4.5 pg/mL, Il-1B: baseline 7.6 ± 0,8 pg/mL vs placebo-treated 7.9 ± 2.2 pg/mL, TNF-α: baseline 12.4 ± 2.1 pg/mL vs placebo-treated 13.3 ± 5.8 pg/mL). Likewise, in the vitamin D3 treated group, there was a higher level of protective markers as serum levels of IL-10, Ang-(1-7) and Ang-(1-7)/Ang II ratio than in placebo group (IL-10: vitamin D3 treated 30.2 ± 12.3 pg/mL vs placebo-treated 27.7 ± 10.4 pg/mL, Ang-(1-7): vitamin D3 treated 1129.1 ± 253 pg/mL vs placebo-treated 864.1 ± 128 pg/mL, Ang-(1-7)/Ang II ratio: vitamin D3 treated 27.9 ± 5.3 vs placebo-treated 18.1± 4.2). Despite this, these changes did not reach statistical significance as a probable consequence of the small sample size. Thus, in the placebo group, none of the pro-inflammatory cytokines (IL-6, IL-8, IL-1B, and TNF-α) decreased by more than 23 %. However, the decrease seemed to be higher among the patients treated with vitamin D, since the variation was between 32 % and 43%.

As in a previous report from our laboratory, we observed that in groups (placebo and vitamin D3 treatments), a rise in Ang-(1-7) and a reduction in Ang II plasma levels occurred. In addition, the Ang-(1-7)/Ang II ratio, as an indirect measure of ACE2 enzymatic activity also increased in both groups. Furthermore, patients treated with vitamin D3 showed a trend towards reduction in all pro-inflammatory interleukins measured [5]. These trends -not statistically significant- could respond to the unexpected basal levels of vitamin D. The main benefits obtained with vitamin D3 supplementation have been obtained when patients had deficient levels of it [6]. In the same way, a meta-analysis of 25 randomized clinical trials confirmed that patients with low vitamin D status gained more protection with the supplementation [7]. Therefore, these patients could be targeted for vitamin D3 treatment because they have a higher potential benefit [8].

Added to the problem of adequate vitamin D levels and a low number of patients who adhered to the study, we found 4 obese and old ones. In this sense, it is central to consider that obesity and aging are two leading causes that fuel the inflammatory process [9,10] and could have interfered with the study results from interpretation. Therefore, we performed an exploratory analysis, excluding patients with a BMI greater than 40 and those older than

70. Additionally, we consider the baseline data of the remaining 11 patients belonging to the same group. We also evaluate the percentage of change from baseline of both groups, placebo and vitamin D3 treated patients. In this sense, we verified a trend to the highest reduction in pro-inflammatory ILs and protective markers of Ang-(1-7) and Ang-(1-7)/Ang II ratio in the vitamin D3 treated group. The results of the CARED-TRIAL sub-study have shown promissory findings, as both Ang-(1-7) and the ratio Ang-(1-7)/Ang-II were more increased in vitamin D3-treated patients (doubling Ang-(1-7) levels and the ratio), compared to placebo. Hence, we can elucidate that the observed increasing trend in these peptides could result from the reduced sequestration of ACE2 in the membrane by SARS-CoV-2 due to the treatment with vitamin D3.

Despite the observed trend, the CARED-TRIAL sub-study showed that acute treatment with high doses of vitamin D3 did not significantly modify the levels of cytokines or Ang II and Ang-(1-7). In this regard, it would be essential to consider that, in addition to the small sample size, all patients who participated in the CARED-TRIAL sub-study had normal plasma vitamin D levels at baseline. Thus, the basal vitamin D plasma levels of the participants could have influenced the results, as the effects of vitamin D administration may not be the same in individuals with vitamin D deficiency/insufficiency as in patients with normal plasma levels of vitamin D. Thus, further studies should be done to clarify this point.

Finally, high-dose vitamin D3 supplementation in mild to moderate COVID-19-positive patients with plasma vitamin D levels within the normal range -even eliminating obesity and ageing- did not cause significant changes in clinical aspects. Still, we observed a trend of interest in specific cytokines and angiotensin's.

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Declaration of competing interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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