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BRIEF ARTICLE

Vitamin D improves viral response in hepatitis C genotype 2-3 naïve patients

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Abstract

AIM: To examine whether vitamin D improved viral response and predicted treatment outcome in patients with hepatitis C virus (HCV) genotype 2-3.

METHODS: Fifty patients with chronic HCV genotype 2-3 were randomized consecutively into two groups: Treatment group [20 subjects, age 48 ± 14 years, body mass index (BMI) 30 ± 6, 65% male], who received 180 μg pegylated α-interferon-2a plus oral ribavirin 800 mg/d (Peg/RBV), together with oral vitamin D3 (Vitamidyne D drops; 2000 IU/d, 10 drops/d, normal serum level > 32 ng/mL) for 24 wk; and control group (30 subjects, age 45 ± 10 years, BMI 26 ± 3, 60% male), who received identical therapy without vitamin D. HCV RNA was assessed by reverse transcription polymerase chain reaction. Undetectable HCV RNA at 4, 12 and 24 wk after treatment was considered as rapid virological response, complete early virological response, and sustained virological response (SVR), respectively. Biomarkers of inflammation were measured.

RESULTS: The treatment group with vitamin D had higher BMI (30 ± 6 *vs* 26 ± 3, $P < 0.02$), and high viral load (> 400 000 IU/mL, 65% *vs* 40%, $P < 0.01$) than controls. Ninety-five percent of treated patients were HCV RNA negative at week 4 and 12. At 24 wk after treatment (SVR), 19/20 (95%) treated patients and 23/30 (77%) controls were HCV RNA negative ($P < 0.001$). Baseline serum vitamin D levels were lower at baseline (20 ± 8 ng/mL) and increased after 12 wk vitamin D treatment, to a mean level of (34 ± 11 ng/mL). Logistic regression analysis identified vitamin D supplement [odds ratio (OR) 3.0, 95% CI 2.0-4.9, $P < 0.001$], serum vitamin D levels (< 15 or > 15 ng/mL, OR 2.2, $P < 0.01$), and BMI (< 30 or > 30, OR 2.6, $P < 0.01$) as independent predictors of viral response. Adverse events were mild and typical of Peg/RBV.

CONCLUSION: Low vitamin D levels predicts negative treatment outcome, and adding vitamin D to conventional Peg/RBV therapy for patients with HCV genotype 2-3 significantly improves viral response.

Key words: Hepatitis C; Genotype 2-3; Vitamin D; Sustained viral response; Peg-interferon alpha 2a

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INTRODUCTION

The current treatment for chronic hepatitis C virus (HCV) infection is pegylated interferon α combined with ribavi-

rin (Peg/RBV), administered for 24 wk for HCV genotype 2 or 3 or 48 wk for HCV genotype 1[1]. The aim of HCV therapy is a sustained viral response (SVR), which is defined as undetectable serum HCV RNA level at 24 wk after cessation of therapy. For patients with HCV genotype 1, the rate of SVR ranges between 38% and 46%[2,3]. For patients with HCV genotype 2 or 3, the rate of SVR ranges between 74% and 77%. In subgroups of this population (e.g., Hispanics and African Americans), the rate of SVR is even lower, reaching only 19% in genotype 1 and 57% in genotype 2 or 3[4]. These differences in the rate of viral response are not explained by baseline viral load or adherence to treatment. Recent efforts to improve patient outcomes have focused on adding new antiviral therapies that specifically target HCV, including polymerase or protease inhibitors[5]. However, few studies have addressed the issue of improving host factors with immunomodulators.

Vitamin D is a potent immunomodulator that favors innate immunity and cell differentiation[6,7]. Increased production of 1,25-dihydroxy vitamin D3 results in the synthesis of cathelicidin, a peptide capable of destroying many viral infectious agents as well as *Mycobacterium tuberculosis*. Low serum levels of 25-hydroxyvitamin D (< 20 ng/mL) prevent macrophages from initiating this innate immune response, which may explain why African Americans, who are often vitamin D deficient, are more prone to contracting viral infections and tuberculosis than Caucasians are[8]. Moreover, vitamin D improves insulin sensitivity[9], suppresses proinflammatory cytokines, increases anti-inflammatory cytokines, and improves CD4 T cell hyper-responsiveness[10]. Vitamin D deficiency is very common (92%) among patients with chronic liver disease, and at least one-third of them suffer from severe vitamin D deficiency (< 12 ng/mL)[11]. Israeli subjects from various ethnic backgrounds are at higher risk of vitamin D deficiency[12]. Petta *et al*[13] have recently shown a low serum vitamin D level to be related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. More recently, we have shown that adding vitamin D to conventional Peg/RBV therapy for naïve, genotype 1 patients with chronic HCV infection significantly improves SVR[14]. Southern *et al*[15] have retrospectively shown that vitamin D supplementation improves the SVR in patients with HCV genotype 2-3, with mild-to-moderate fibrosis.

The aim of the present study was to assess prospectively the influence of vitamin D supplementation on outcome (SVR) in the treatment of patients with chronic hepatitis C with HCV genotype 2-3.

MATERIALS AND METHODS

Subjects

Study inclusion criteria were age 18-65 years, a chronic genotype 2 or 3 HCV infection, no previous treatment for hepatitis C, seronegative for hepatitis B virus, hepatitis A virus, and HIV infection, an absolute neutrophil count of > 1500/mm^3, a platelet count of > 90 000/mm^3, and a normal hemoglobin level. Liver biopsies were not required prior to study entrance. Exclusion criteria were decompensated liver disease (cirrhosis with Child-Pugh score > 9), another cause of clinically significant liver disease, or the presence of hepatocellular carcinoma. The protocol was approved by the two institutional review boards of both study centers (Ziv Medical Center, and Hilel Yafe Medical Center, Israel), and all patients provided written informed consent to participate in the study.

Study design and organization

This was an intention to treat prospective randomized study. Fifty consecutive chronic hepatitis C (genotype 2-3) treatment-naïve patients were stratified according to ethnic group (i.e., Russian/Jewish/Arab) due to possible difference in vitamin D levels, and alanine aminotransferase levels were enrolled. They were consecutively and randomly assigned to one of two study groups. The treatment group comprised 20 patients [mean age 48 ± 14 years, body mass index (BMI) 30 ± 6, 65% male], who received pegylated interferon α2a (180 μg once weekly by subcutaneous injection) plus ribavirin orally at a dose of 800 mg/d, with vitamin D [Vitamidyne 2000 IU/d (Fischer, Israel), 10 oral drops, target serum level > 32 ng/mL] for 24 wk. The control group of 30 patients (mean age 45 ± 10 years, BMI 26 ± 3, 60% male) received pegylated interferon α2a (180 μg once weekly) plus ribavirin (800 mg/d) without vitamin D for 24 wk. Vitamin D3 was given by oral drops at a dose of (2000 IU/d, 10 drops) to the treatment group for 12 wk before the initiation of antiviral treatment until serum levels reached > 32 ng/mL. The adherence to vitamin D treatment was excellent during the entire course and the vast majority of patients in the treatment group achieved the target level. Vitamin D supplement was maintained during the course of therapy and during follow-up.

Efficacy assessments

Plasma HCV-RNA levels were measured using the COBAS Taq Man HCV assay, version 1.0 (Roche Molecular Systems, Israel), with a lower limit of quantification of 50 IU/mL and a lower limit of detection of 10 IU/mL. HCV-RNA levels were measured at the time of screening and during the treatment period at weeks 4, 12 and 24 for the treatment group, and at 24 wk after treatment. HCV RNA was not measured at weeks 4 and 12 in the control group because this was not included in the health package at the time of study. Subjects had a safety follow-up visit after the completion of treatment. Those who had undetectable HCV RNA levels at the time of treatment completion had follow-up visits 24 wk later, at which time, HCV-RNA levels were measured again. Assessment of efficacy was SVR, i.e., undetectable HCV RNA at 24 wk after treatment. Clearance of HCV RNA was assessed at week 4 (rapid virological response), week 12 (complete early virological response), and at week 24 at the end of treatment response (ETR) for the treat-

Table 1 Baseline demographic, clinical, and virological characteristics of all patients

Baseline demographics	Peg/RBV (n = 30)	Vitamin D + Peg/RBV (n = 20)	P value
Age (yr)	45 ± 10	48 ± 14	0.3
Males (%)	60	65	0.2
BMI (kg/m^2)	26 ± 3	30 ± 6	0.02
HCV virus genotype: 2/3	17/13	11/9	0.01
SVR rate genotype: 2/3	90%/64%	100%/89%	0.01
Vitamin D (ng/mL)	19 ± 6	20 ± 8	0.1
Week 12 baseline	-	34 ± 11	
Baseline HCV RNA > 400 000 IU/mL)	40%	65%	0.01
Baseline ALT (U/L)	50 ± 16	48 ± 10	0.1
Ethnicity (Russian/ Jewish/Arabic)	26/3/1	16/2/2	0.1
CRP	0.5 ± 1.0	0.4 ± 0.5	0.3

BMI: Body mass index; HCV: Hepatitis C virus; SVR: Sustained viral response; ALT: Alanine aminotransferase; CRP: C-reactive protein; Peg/RBV: Pegylated interferon α combined with ribavirin.

ment group. Patients with ETR who tested positive for HCV RNA during follow-up were classified as relapsers. Breakthrough was defined as an increase in HCV RNA level of 1 $\log_{10}$ unit, as compared with the lowest value[3].

Safety assessment

Biochemical assessments were performed at each visit during the treatment period and after treatment a follow-up visits. Data on adverse events were collected and physical examinations were also performed each time. The safety assessment included complete blood count, antinuclear antibody and thyroid stimulating hormone levels. Pegylated interferon α2a was reduced to 90 μg/wk in patients with neutrophil count < 750 cells/μL, and withdrawn temporarily in patients with counts < 500 cells/μL. The same dose reduction was applied if platelet levels fell under 50 000 cells/mm^3, with pegylated interferon being discontinued when the 25 000 cell/mm^3 threshold was reached. In both treatment arms, the ribavirin dose was tapered by 200 mg/d in patients with hemoglobin < 10 g/dL, and discontinued altogether in patients with hemoglobin level < 8.0 g/dL.

Clinical and laboratory measurements

25(OH) vitamin D3 levels were determined by ^{125}I radioimmunoassay (Dia-Sorin, Stillwater, MN, United States)[16]. 25-OH vitamin D is the major circulating form of vitamin D and is used as an indicator of vitamin D status. Vitamin D deficiency was defined as a 25(OH) D serum level < 12 ng/mL, vitamin D insufficiency as 25(OH) D level 12-32 ng/mL, and vitamin D sufficiency as > 32 ng/mL[12]. BMI was calculated as weight in kilograms divided by the square of height in meters [17]. Obesity was defined as BMI > 30 kg/m^2. C-reactive protein (CRP) was determined by the nephlometric method[18]. Thyroid stimulating hormone, antinuclear antibody, glucose, liver enzymes, albumin, bilirubin, prothrombin time, and creatinine were measured by standard biochemical tests.

Statistical analysis

Results were expressed as the mean ± SD. The difference between two groups was assessed by χ^2 test for categorical variables and by Mann-Whitney rank for continuous variables. The Spearman correlation was used to express correlations between variables. The primary study endpoint was evidence of the influence of vitamin D on viral response at week 24 after treatment. Logistic regression analysis was used to document independent variables that predicted SVR. The significance level was set at $P < 0.05$. The statistical analysis was carried out with the WINSTAT software program (Kalmia, San Diego, CA, United States).

RESULTS

Twenty percent of the patients in the treatment group had severe baseline vitamin D deficiency (< 12 ng/mL), 60% showed insufficiency, and 20% had sufficient vitamin D levels. In the control group, 30% of the patients had baseline vitamin D deficiency, 50% had insufficiency, and 20% had sufficient vitamin D levels. Table 1 shows the clinical and biochemical parameters of the patient populations. The treatment group with vitamin D had higher BMI (30 ± 6 *vs* 26 ± 3, $P < 0.02$), and high viral load (> 400 000 IU/mL, 65% *vs* 40%, $P < 0.01$) than patients in the control group. There were no significant differences between the groups in terms of age, HCV genotype, ethnic background, aminotransferases, or CRP levels. Figure 1 shows the rates of viral response in the treatment and control groups: 19/20 (95%) patients in the treated group were HCV-RNA negative at weeks 4 and 12. At 24 wk after treatment (SVR), 19/20 (95%) patients in the treatment group and 23/30 (77%) in the control group were HCV RNA negative ($P < 0.001$). The rate of viral breakthrough and relapse was nill. The rates of non-response were significantly lower in the treatment group compared to the control group [1/20 (5%) *vs* 7/30 (23%), $P < 0.001$]. Figure 2 shows the baseline and week 12 vitamin D levels in the treatment group before the initiation of antiviral therapy. Serum vitamin D levels were significantly lower at base line (20 ± 8 ng/mL) and increased after 12 wk of vitamin D treatment to a mean level of 34 ± 11 ng/mL. Adherence to vitamin D treatment was excellent during the entire course, and all patients in the treatment group achieved the target level. Vitamin D supplementation was maintained during the course of therapy with the same amount (2000 IU/d) as in the lead in phase.

Predictive factors for SVR in patients treated with Peg/RBV combination therapy are shown in Table 2. Logistic regression analysis identified vitamin D supplementation (OR 3.0, 95% CI 2-4.9, $P < 0.001$), serum vitamin D levels (< 15 or > 15 ng/mL; OR 2.2, $P < 0.01$) and BMI (< 30 or > 30, OR 2.6, $P < 0.01$) as independent predictors of viral response. Thus, vitamin D

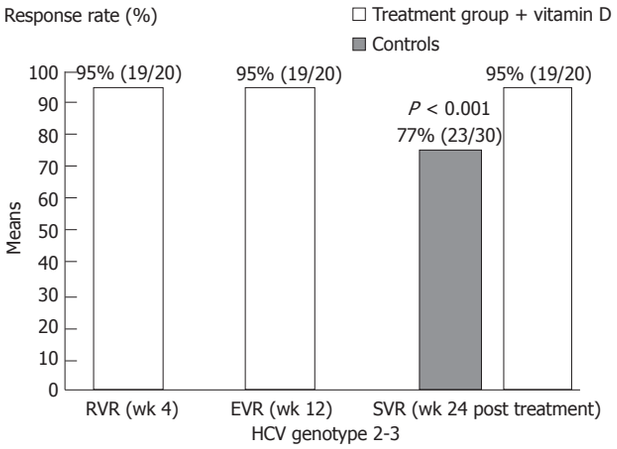

Figure 1 Rate of rapid virologic response, early virologic response and sustained viral response in the treatment (*n* = 20) and control (*n* = 30) groups. Rapid virologic response (RVR) was defined as undetectable hepatitis C virus (HCV) RNA at 4 wk during treatment. Early virologic response (EVR) was defined as undetectable HCV RNA at 12 wk during treatment. Sustained viral response (SVR) was defined as undetectable HCV RNA at 24 wk after cessation of therapy.

Table 2 Viral response, vitamin D levels and biomarkers of inflammation, insulin resistance and oxidative stress in all patients

	OR	95% CI	*P* value
Received vitamin D supplementation (yes *vs* no)	3.0	2.0-4.9	< 0.001
Base line vitamin D (< 15 or > 15 ng/mL)	2.2	1.1-4.3	0.01
Genotype (2 *vs* 3)	2.0	1.2-3.8	0.01
High viral load (< 400 000 or > 400 000 IU/mL)	2.8	1.2-4.0	0.001
Baseline CRP (< 0.5 or > 0.5 mg/dL)	1.0	0.5-1.9	0.5
BMI > 30 (kg/m^2) (yes *vs* no)	2.6	0.8-3.5	0.01

CRP: C-reactive protein; BMI: Body mass index; OR: Odds ratio.

supplementation emerged as being more responsible for higher SVR than the baseline vitamin D level. The SVR rate was significantly different between patients with HCV genotype 2/3 in the treated group versus those in the control group (100%/89% *vs* 90%/64%, $P < 0.01$).

The most common adverse events were mild, similar in both groups, and consistent with typical interferon–ribavirin induced systemic symptoms including nausea, headache, insomnia, myalgia, pyrexia, mild neutropenia, thrombocytopenia, and mild anemia. No serious adverse events were seen. Adherence to Peg/RBV therapy was excellent and there was no difference in dose reduction for Peg/RBV therapy due to adverse events in both groups. No patients discontinued treatment. Changes in laboratory values during the study were consistent with those reported in association with the combined use of Peg/RBV[3].

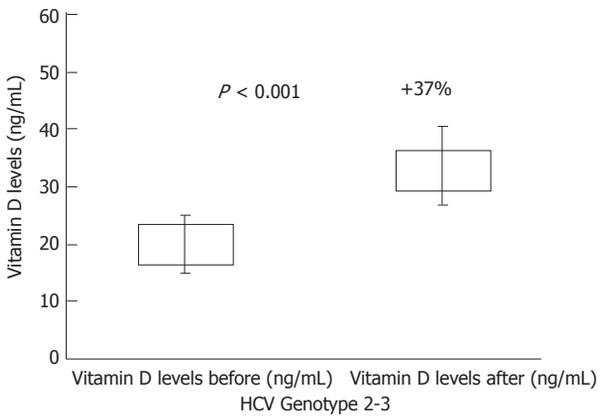

Figure 2 Vitamin D serum levels before and 12 wk after initiation of antiviral treatment (*n* = 30) and vitamin D supplementation (*n* = 20). Percentage change was +37%.

DISCUSSION

The results of this study suggest that the addition of a vitamin D supplement to current standard therapy can significantly improve the rate of SVR in treatment-naïve patients with HCV genotype 2-3, compared to the rates with standard therapy alone. The observed SVR rate in the control group (77%) is consistent with previous reports[2,3]. The overall responses reflect a marked increase in the rate of virological response at week 24 after cessation of therapy (95% *vs* 77%) and a low rate of non-response (5% *vs* 23%) with vitamin-D-based treatment, as compared to the control group[19].

There are only two reports dealing with the association between vitamin D status and outcome of antiviral therapy for chronic HCV viral infection. Petta *et al*[13] have retrospectively analyzed a cohort of 167 patients treated with peg-interferon and ribavirin for hepatitis C, and detected an association between lower vitamin D serum levels and failure to achieve SVR. Our results provide further support to these data. The second study by Bitetto *et al*[20] has shown that vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C in liver transplant recipients. Several differences between those two studies should be noted. Their HCV patients were immunocompromised and they were supplemented by low-dose vitamin D (800 IU/d) after liver transplantation. In addition, most of their HCV patients (75%) had low vitamin D levels despite treatment. Finally, that study was retrospective and focused on the prevention of osteoporosis and not on the treatment of hepatitis C. Very recently, Southern *et al*[15] have shown the beneficial effect of vitamin D supplementation on the outcome in patients with chronic HCV genotype 2-3 infection. However, this study was retrospective and the authors used Calcichew D3 Forte during the course of treatment without indicating the dose or serum levels of vitamin D.

The exact mechanism of action leading to improved

response to antiviral treatment is unknown in patients receiving vitamin D. Vitamin D is metabolized by the liver and is converted to 1,25 dihydroxyvitamin D3, which is the active form of the vitamin[6,7]. Those with chronic liver disease may have poor conversion from vitamin D3 or any of its other biologically active metabolites[11]. 1,25 vitamin D3 appears to modulate immunity principally via regulating T-cell function[21]. The vitamin D receptor (VDR) is expressed on virtually every type of cell involved in immunity[22]. The immunomodulatory actions of vitamin D are elicited through its direct action on T-cell antigen-presenting cell function[23]. T helper cell type 1 (Th1) cell actions are intensified when vitamin D is insufficient, as in the majority of our patient population, or when signals through VDR are weak. Regulatory T cells and Th2 cells are diminished, thus favoring an autoimmune Th1 response[24]. This is a proinflammatory response that may impair interferon and insulin signaling, thus decreasing viral response[25,26]. A recent study, comprised of 120 patients with chronic infection with HCV genotype 1 reported a Th1 to Th2 ratio < 15.5 (OR, 9.6) was significantly associated with SVR[27]. The overall effect is a switch from the Th1/Th17 response to the Th2/Treg profile[28]. However, Th1 and Th2 measurement was not performed in the present study. Persistent HCV infection modulates the balance between immunostimulatory and inhibitory cytokines that can prolong inflammation and lead to fibrosis and chronic liver diseases[29]. More recently, Gutierrez *et al*[30] have shown that vitamin D3 increases VDR protein expression and inhibits viral replication in cell culture.

It is well known that people of African and Hispanic descent are less likely to respond to standard therapy[31]. This may be due to a polymorphism of the *IL28B* gene and to vitamin D deficiency[13,32]. The vast majority of subjects in the present study had vitamin D insufficiency that was possibly related to low exposure to the sun and/or to a low supply of vitamin D from the diet. Recently, an important study by Lange *et al*[33] has confirmed the association of response to therapy with vitamin D levels and, even more significantly, by describing novel associations between genetic polymorphisms within VDR; especially in the α-hydroxylase promoter region (CYP27B1-1260), and vitamin D levels in HCV patients. The authors showed also that fibrosis alone is not the key to understanding the impact of chronic hepatitis C on vitamin D metabolism.

The role of insulin resistance and supplementation of vitamin D with regards to chronic HCV infection has been investigated previously by our group[14]. Insulin resistance has emerged as one of the most important host factors in the prediction of response in non-diabetic HCV-infected patients treated with Peg/RBV, and is a common denominator to the majority of features associated with difficult-to-treat patients[34]. Vitamin D is also known to help prevent type 2 diabetes and it is possible that low levels of vitamin D lead to insulin resistance[9]. The direct effect of vitamin D may be mediated by binding of its circulating active form to the pancreatic B cell VDR[35,36]. Moreover, oxidative stress leaches calcium, and vitamin D helps absorb calcium[37]. In the current study, increasing levels of vitamin D to > 32 ng/mL increased the response to antiviral therapy. The calcium levels were normal in our patient population.

Multivariate analysis revealed that vitamin D supplementation, baseline vitamin D levels, viral load, and hepatitis C genotype remained as independent predictors. Thus, it can be concluded that vitamin D supplementation is responsible for a higher SVR, rather than the baseline vitamin D level. Limitations of the present study include the small number of patients, and the lack of Th1 and Th2 immune response. The identification of determinants of response such as polymorphisms of the *IL28B* gene and within the VDR may explain the difference in response rates between patients with different ethnic backgrounds. This was not done in our study because data on *IL-28B* and VDR polymorphism were not available. Because of the small number of patients in the present study, a multicenter study with a larger number of patients is warranted. Another limitation was the lack of results that documented the viral response at weeks 4 and 12 in the control group. This test was not included in the health package at the time of study recruitment. Finally, we did not have a dose-response relationship for vitamin D supplementation. The dose of 2000 IU/d was based on previous investigations[38].

In conclusion, the addition of vitamin D to pegylated interferon α2b and ribavirin in naïve patients infected with HCV genotype 2-3 significantly increased the rate of viral response. We suggest routine testing of vitamin D levels prior to combination therapy and replacement during treatment for chronic hepatitis C.

COMMENTS

Background

Treatment of patients with chronic hepatitis C virus (HCV) infection with pegylated interferon + ribavirin (Peg/RBV) achieves virus clearance in < 50% of cases. Thus, there is a need to find new medication.

Research frontiers

Recent efforts have focused on adding new antiviral therapies including polymerase or protease inhibitors to standard therapy. However, these drugs have many side effects like rash and are very expensive. Few studies have addressed the issue of improving the immune system of the patient with immunomodulators, such as vitamin D supplementation.

Innovations and breakthroughs

Adding vitamin D supplements (2000 IU/d) to standard Peg/RBV therapy for patients with HCV genotype 2-3 significantly improves viral response (viral clearance) from 42% to 86% for genotype 1 and from 77% to 95% for genotype 2/3. The addition of vitamin D is cheap and without side effects.

Applications

The authors suggest routine testing of vitamin D levels prior to Peg/RBV therapy and adding vitamin D supplementation for chronic hepatitis C until reaching a blood level > 32 ng/mL.

Terminology

Response rate: Undetectable virus in the blood at 6 mo after discontinuation of therapy; peg-interferon is an immune system modulator that improves immune function and clears virus; ribavirin is a drug that inhibits viral replication.

Peer review

This is an interesting study and supports the previous work from this group on HCV treatment in genotype 1 subjects.

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