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Original article

Vitamin D levels and chronic hepatitis C

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SUMMARY

Background and purpose: Vitamin D (VitD) is involved in homeostasis of calcium and interacts with parathyroid hormone (PTH). Low levels of VitD in chronic liver diseases, in particular in chronic hepatitis C (CHC) was reported. We aimed to determine the levels of VitD and PTH in patients with CHC without cirrhosis to evaluate the disturbance of VitD-PTH axis.
Methods: We conduced a case–control study enrolling 59 patients with CHC and 59 controls. We determined serum concentration of VitD, PTH, calcium and phosphate. VitD was quantified by chemiluminescence immunoassay. PTH was measured by 2-site chemiluminescent enzyme-labeled immunoassay.
Results: The mean value of VitD was 26.28 and 28.43 ng/ml in HCV patients and controls respectively ($p < 0.31$). The distribution of the severity of VitD deficit in HCV population was the following: 5% had a deficiency, 64% had an insufficiency and 31% had normal levels. No difference was observed in the control group ($p < 0.9$). The mean value of PTH was 17.04 and 26.7 pg/ml in HCV patients and controls respectively ($p < 0.0004$). Calcium and phosphate were in the range of normality in both.
Conclusions: The VitD deficit is similar in HCV-patients and general population of the same geographic area. Therefore we can state that this is a public health problem.

1. Introduction

Vitamin D (VitD) is involved in calcium homeostasis and its deficiency in adults leads to osteopenia and osteoporosis.[1–5] In addition, it has been documented that VitD plays a series of non-classical actions such as anti-proliferative, pro-differentiation, pro-apoptotic, anti-inflammatory, and immunoregulator activity.[6,7] As a consequence, a large number of diseases has been associated with VitD deficiency.[8,9]

VitD deficiency is recognized as a worldwide problem with consequences on global health.[4] In the hepatological setting, VitD deficiency has been associated with: cholestatic affections, due to the concomitant malabsorption,[10] alcohol related liver disease, due to an inadequate intake,[11] and cirrhotic status due to the impaired liver function and reduced mitochondrial 25-alpha-hydroxylation.[12] More recently, it has been reported that serum VitD deficiency is more prevalent in patients with chronic hepatitis C (CHC),[13] and this finding has been mainly related to the HCV etiology rather than to the severity of liver disease.[10]

Current knowledge has not definitively clarified the correlation between HCV infection and levels of VitD. Few data are available regarding the fact that low levels of VitD promote a negative evolution of CHC or, vice versa, that HCV infection leads to depletion of VitD levels through a mechanism that remains unclear.[14,15]

This information is crucial because VitD is a modulator agent of the innate and adaptive immune response which seems to be involved in spontaneous and interferon induced HCV clearance. Preliminary data suggest that low levels of VitD are predictors of bad response to antiviral therapy with PEG-IFN and ribavirin, and that the VitD supplementation increases the percentage of patients with sustained virological response (SVR).[16–18]

In fact, literature lacks data on 25(OH)D_3 mean levels in a well selected population with CHC without cirrhosis and biochemical signs of cholestasis.

Furthermore, it is essential to point out that serum levels of VitD depend on many factors such as age, sun exposure, skin color, use of

Abbreviations: VitD, vitamin D; CHC, chronic hepatitis C; PTH, parathyroid hormone; CVs, coefficients of variation.

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protective factors for UVB radiation, obesity and VitD dietary intake and PTH levels.[19–21] Therefore, the large number of potential confounders determines a great intra- and inter-individual variability in serum levels of VitD and complicates the accurate assessment in a non homogeneous population.

Finally, it is important to consider that the most recent papers regarding the relationship between VitD and liver damage related to HCV infection have not assessed the main variables involved in VitD metabolism (VitD - PTH axis) and this aspect could affect the interpretation of the results.[19]

The aims of the present study were: 1. to determine the 25(OH)D_3 mean levels in a population of adult patients with CHC without cirrhosis and no signs of cholestasis compared to a well matched healthy population of the same geographical area; 2. to measure the main parameters regulating VitD metabolism, especially the calcium and phosphate levels and the activation of PTH feed-back; 3. to evaluate the dietary intake of VitD in patients and controls.

2. Patients and methods

2.1. Patients

From 22/1/2009 to 29/4/2010, 59 consecutive adult patients with CHC residing in Southern Italy and fulfilling the inclusion and exclusion criteria detailed later, were recruited at the Gastrointestinal and Liver Unit at the University Hospital of Naples "Federico II". The diagnosis was done on the basis of biochemical and clinical parameters, according to current international guidelines. In 29 out of 59 patients a liver biopsy was performed and staging of CHC was assessed according to the Ishak score.[22]

The inclusion criteria were: age >18 years, diagnosis of CHC, baseline creatinine values within the normal range and compensated liver disease. The exclusion criteria were: liver disease etiology different from HCV or mixed causes, cirrhosis status, alcohol intake >20 g/day, drug abuse, serum positivity for HIV, eating disorders, intestinal disease or disease associated with malabsorption, malnutrition and signs of cholestasis (increase serum levels of GGT, bilirubin and/or alkaline phosphatase). Therapy with medications known to affect VitD_3 metabolism (calcium, VitD supplementation, hormonal therapy, alendronate) were also excluded.

Fifty-nine adult healthy controls with no history of liver disease were included as controls (controls were recruited among patient's kin and Hospital staff). All patients and controls were Caucasian and were living in Southern Italy. Moreover, the same exclusion criteria used for patients were applied to controls.

Demographic characteristics and clinical parameters at baseline were recorded for all cases and controls.

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices.

Approval was obtained from the Institutional Review Board and Ethics Committee and a written informed consent was obtained from all cases and controls.

2.2. Vitamin D and PTH measurements

Blood samples were collected in winter/spring from 38 patients and 26 controls, in summer/autumn from 21 patients and 33 controls.

The serum was immediately separated by centrifugation and stored at −80 °C for the subsequent assay of VitD and PTH.

Total 25-hydroxyvitamin (25(OH)D_3) was quantified by a direct competitive chemiluminescence immunoassay (Liaison®, DiaSorin, Turin, Italy), with a specificity of 100% for 25(OH)D_3. The analytical measurement range of detection is 4–150 ng/ml, whereas the intra-assay coefficients of variation (CVs) were 5.5%, 2.9%, and 4.8% and the inter-assay CVs were 12.7%, 6.9%, and 7.9% for low, medium, and high points of the standard curve, respectively. According to manufacturer's instruction, serum concentrations of <10 ng/ml 25(OH)D3 were defined as severe VitD deficiency, <30 ng/ml 25(OH)D3 as VitD insufficiency, whereas a range of 30–100 ng/ml 25(OH)D3 was considered as normal.

On the same serum samples intact PTH was measured by 2-steps chemiluminescent immunoassay for the 1–84 amino acid chain without cross-reactivity with the 7-84 PTH fragment on the Liaison auto-analyzer (Liaison®, DiaSorin, Turin, Italy). Analytical sensitivity was ≤1.7 pg/ml, whereas the intra-assay CVs were 5.9%, 3.0%, and 3.9% and the inter-assay CVs were 9.0%, 5.4%, and 5.6% for low, medium, and high points of the standard curve, respectively.

2.3. Dietary interview

A dietary interview was given to patients and controls in order to analyze their eating habits and VitD intake. The interview was conducted using an image-based computer program able to make a semi-quantitative assessment of dietary intake. This program provides an appropriate estimate of nutrient intake in relation to weight, height, sex and age. Each subject was asked to indicate the frequency and quantity of intake of 60 foods belonging to the following groups: cereals, milk and dairy products, fish, meat and eggs, cold cuts, fruits and vegetables etc., drinks (coffee, tea, fruit juices, wine, sugary drinks).

Since there are no specific data on the optimal VitD dietary intake for the Italian population, we referred to the data of NHANES II (National Health and Nutrition Examination Survey II) to estimate the necessary intake levels of VitD that should not be less than 15 μg/day. The same data base recommended dietary intakes of 1000 mg/day for calcium and phosphate.[23]

2.4. Statistical analysis

The results were expressed as median and range or mean ± Standard Deviation (SD), as appropriate. The differences in percentages were evaluated with the test χ^2. The differences in mean values were evaluated with the *T* Student's test. The probability value <0.05 was considered statistically significant. Data were analyzed using the Stata® Statistics/Data Analysis, StataCorp LP, Texas, USA.

3. Results

3.1. Patients features

Baseline characteristics of patients and controls are summarized in Table 1. Patients and controls were well matched for age and male/female ratio, and BMI was in the normal range in both groups. Among female patients and controls 15/27 and 13/41 were in menopause. No patient showed histological, clinical, biochemical and ultrasonographic signs of liver cirrhosis. In the patients with liver biopsy, the staging of liver fibrosis was <S3, according to the Ishak score.[22]

3.2. 25(OH)D_3 and PTH

The mean value of 25(OH)D_3 was 26.28 and 28.43 ng/ml in HCV patients and controls, respectively (Fig. 1).

As reported in Fig. 2, the distribution of the severity of 25(OH)D_3 deficit was similar in patients and controls. In the HCV population, 5% shows a severe deficiency, 64% insufficiency and 31% normal levels. In controls we observed a severe deficiency in 2% of subjects, an insufficiency in 54% and normal levels in 44% ($p = 0.9$). The