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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

Table 1
Basal characteristics of patients and controls.

	Patients		Controls		*p* Value
N. patients	59		59		–
Gender	*n*	%	*n*	%	
Male/female	32/27	54/46	18/41	30/70	0.061
Age yrs					
Median (range)	54	(28–72)	50	(17–84)	0.083
BMI					
Median (range)	25.60	(15.24–31.25)	22.4	(15–32.89)	0.074
ALT UI/l					
Mean ± SD	78.69 ± 55.2		20.1 ± 4.06		–
AST UI/l					
Mean ± SD	54.45 ± 2.4		21.4 ± 3.89		–
γGT UI/l					
Mean ± SD	57 ± 63		52.3 ± 35.7		0.312
ALP UI/l					
Mean ± SD	81.27 ± 36.12		64.5 ± 19.4		0.094
Albumin mg/dl					
Mean ± SD	4.26 ± 0.25		4.43 ± 0.47		0.723
Bilirubin mg/dl					
Mean ± SD	1.12 ± 0.9		0.83 ± 0.41		0.071
INR					
Mean ± SD	1.07 ± 0.4		1.05 ± 0.3		0.856
Platelets/mm^3					
Mean ± SD	274.000 ± 70118.95		312.000 ± 56000.10		0.093
Creatinine mg/dl					
Mean ± SD	0.90 ± 0.20		0.85 ± 0.12		0.235
HCV genotype					
1a	4%		–		
1b	61%		–		
2	26%		–		
3	5%		–		
4	4%		–		
HCV RNA UI/ml					
Mean ± SD	$2.75 \times 10^6 \pm 3.69 \times 10^6$		–		
Staging					
Unknown	30 (50.8%)		–		
F0–F1	17(58.6%)		–		
F2–F3	12 (51.4%)				
>F3	0 (0%)				

levels of VitD did not show any difference according to sex and menopause state.

In relation to the season of blood collection, the mean value of VitD in patients was 21.52 ± 11.57 ng/ml in spring, 35.12 ± 3.8 ng/ml in summer, 33.16 ± 12.54 ng/ml in autumn and 23.02 ± 11.95 ng/ml in winter. In controls the mean value of VitD was 18.1 ± 8.57 ng/ml in spring, 30.9 ± 6.61 ng/ml in summer, 30.5 ± 11.41 ng/ml in autumn and 29.8 ± 13.14 ng/ml in winter. In the patient group there was a statistical difference between VitD serum levels in the summer/autumn seasons respect to winter/spring ($p = 0.005$). No statistically significant seasonal variation was observed in the control group.

The mean value of PTH was 17.04 and 26.7 pg/ml in HCV patients and controls, respectively ($p = 0.0004$). The percentage of patients with increased PTH levels was 5% in HCV patients and 22.5% in controls ($p = 0.7$). As reported in Fig. 3, an inverse correlation between PTH and VitD has been observed in the two populations ($r = -0.36$ for patients and $r = -0.1$ for controls).

3.3. Calcium and phosphate

Calcium and phosphate were in the normal range in both. The mean levels of calcium were 9.6 mg/dl and 9.2 mg/dl in patients and control, respectively ($p = 0.5$), the ones of phosphate were 3.6 mg/dl and 4 mg/dl, respectively ($p = 0.8$).

3.4. Vitamin D, calcium and phosphate dietary intake

The interview regarding eating habits was given to 53 of the 59 patients and to 38 of 59 controls. The mean dietary intake of VitD was 9.7 ± 19.34 μg/day and 12.25 ± 29.01 μg/day in patients and controls, respectively ($p = 0.007$). The patients with severe deficiency of $25(OH)D_3$ had a mean VitD dietary intake of 1.55 ± 1.59 μg/day, the ones with insufficiency of 9.03 ± 18.77 μg/day and the ones with normal levels of 12.67 ± 22.08 μg/day (Fig. 4). The calcium and phosphate intake in patients was 1052 ± 324.7 mg/day and 1062 ± 478.96 mg/day, respectively, while in controls were 920 ± 269.09 mg/day and 1598 ± 305.43 mg/day ($p = 0.03$).

4. Discussion

Recent findings have indicated that VitD deficiency is more prevalent in patients with CHC. Furthermore, low serum levels of $25(OH)D_3$ in genotype 1 HCV patients have been associated to severe liver fibrosis and low percentage of rapid and sustained virological response.[14] It has been hypothesized that VitD inhibits HCV virus production by a direct antiviral molecular mechanism,

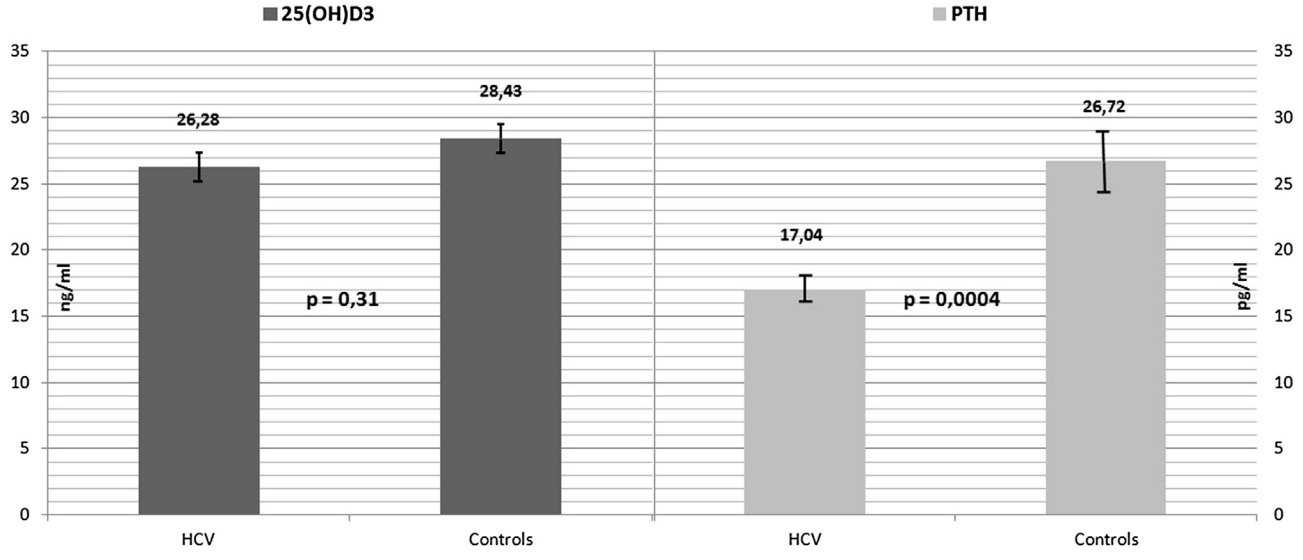

Fig. 1. Mean levels of $25(OH)D_3$ and PTH.

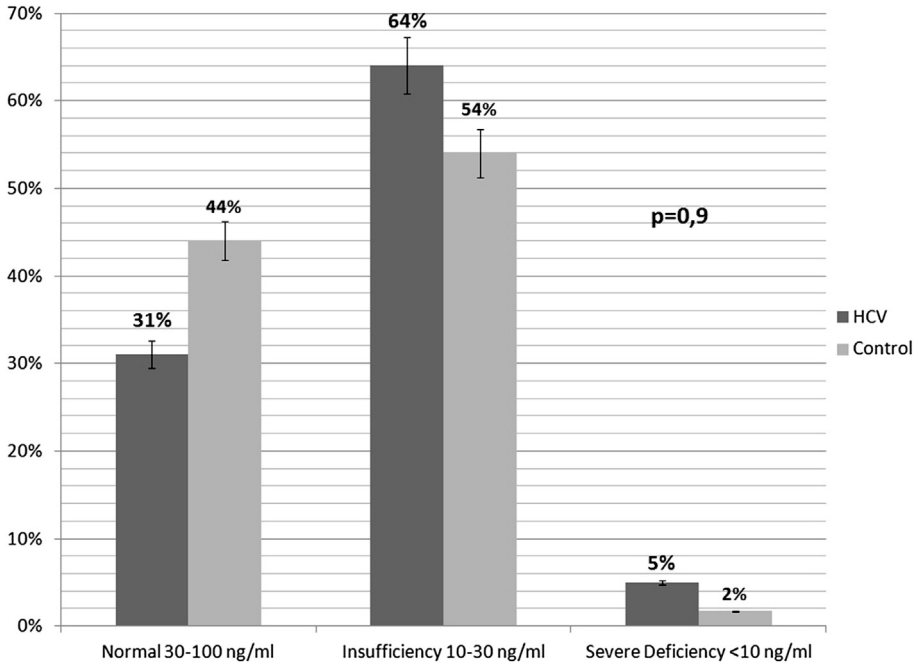

Fig. 2. Severity of 25(OH)D_3 deficiency in patients and controls.

involving the induction of the interferon signaling pathway.[24] Nonetheless, the strict association between deficiency of VitD and HCV infection is still not convincing enough. In fact, serum levels of VitD depend on many variables and no study has taken account of the main factors that influence it.

In our study we analyzed serum 25(OH)D_3 levels in a well selected population of patients with CHC without cirrhosis and signs of cholestasis in comparison to controls. Doubtless the sample size of our study is not very high. The reason is linked to the restrictive inclusion and exclusion criteria used for the selection of patients to eliminate all confounding factors that could influence VitD levels. The main result of our study is the confirmation of low levels of VitD in the population of patients. However, contrary to the findings of other authors, the control population showed a 25(OH)D_3 deficiency similar to that observed in the patient group. No difference was observed when we compared the average values and the percentage of subjects with severe deficit. Although we expected a 25(OH)D_3 deficit in the patient population, we did not expect the same result in the control group. In fact, the control subjects were carefully selected among people without conditions that are associated with low serum 25(OH)D_3 levels and living in southern Italy, an area with adequate sun exposure. The simplest explanation for this result is that inadequate serum levels of VitD represent a problem unlimited to certain pathological conditions such as HCV related liver disease, interestingly, a phenomenon extended to the entire population. It is well known that there is a deficiency of VitD in the northern regions where VitD prophylaxis is customary. Recent studies have shown that VitD deficiency represents a worldwide problem; it is common in Australia, Middle East, India, Africa and South America.[3] In the United States, more than 50% of Hispanic and African-American adolescents in Boston[25] and 48% of white preadolescent girls in Maine had 25(OH)D_3 below

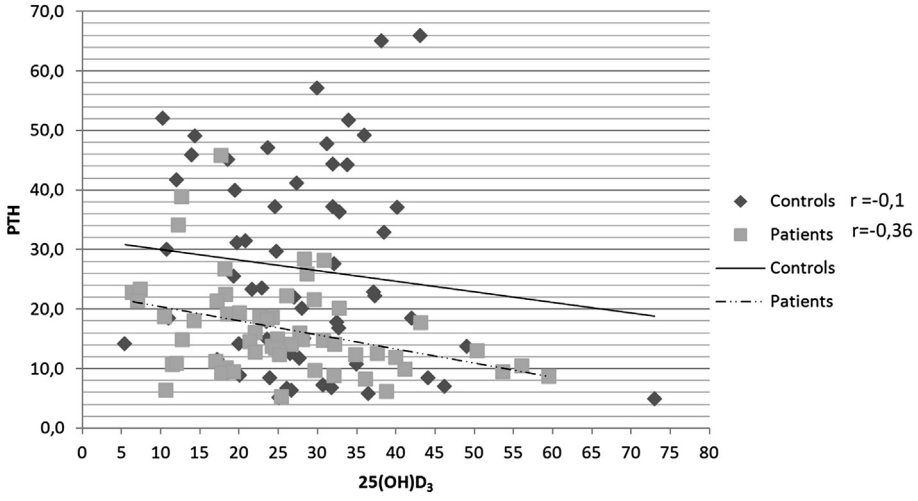

Fig. 3. Relation between 25(OH)D_3 and PTH in patients and controls.

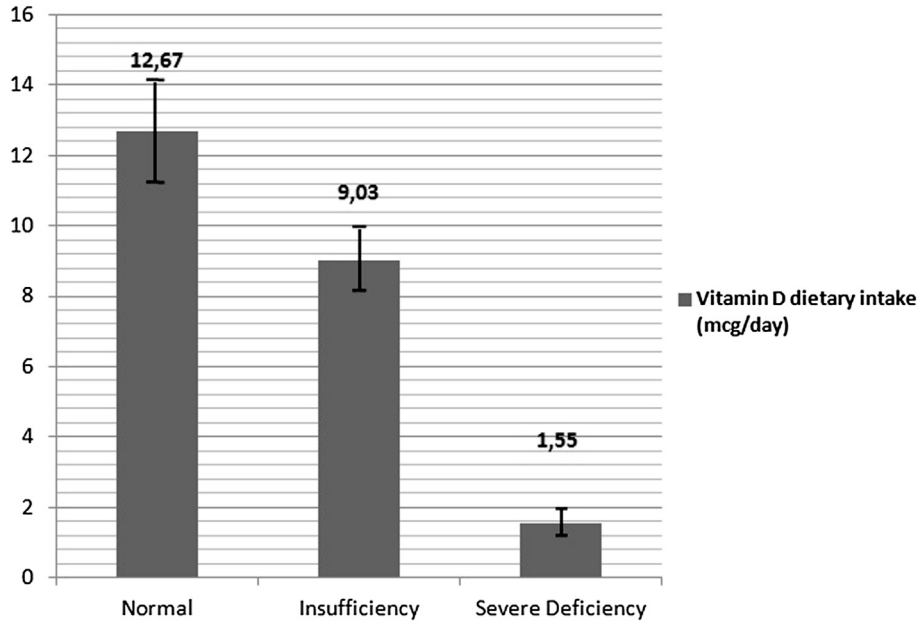

Fig. 4. Relation between dietary intake of vitamin D and serum levels of $25(OH)D_3$ in patients.

20 ng/ml.[26] In a study based on data from the NHANES III, the prevalence of $25(OH)D_3$ deficiency and insufficiency among adolescents and adult population of the United States was reported to be 1% and 58%, respectively.[27]

The major source of VitD is exposure to natural sunlight.[3] Nonetheless, severe deficiency of VitD has also been reported in sunny geographical areas. Two different studies were performed in Iran and Arizona: a severe deficiency was revealed in the 26.9% and in 2% of the subjects, respectively.[28,29]

Therefore, the assumption that living in sunny areas protects people from VitD deficiency is unacceptable. In Italy the reduction of sun exposure can be attributed to a reduced period of holidays, and to the large use of photoprotective skin agents from melanoma. In fact, wearing sunscreen with 30 proof sun protection reduces VitD synthesis in the skin by more than 95%.[23] It would be optimal to have regular daily sun exposure, even of short duration, rather than an occasional weekly exposure. It has been reported that serum levels of VitD are influenced by the season: higher levels in summer/autumn and lower in winter/spring. Our results confirm that serum levels of VitD are higher in patients with CHC during the summer/autumn than in the winter/spring. Differently, the seasonal variability has not been observed in controls. The different seasonal behavior between patients and controls is not easily explainable, even though the unbalanced number of samples analyzed may have influenced the result (38 patients vs 26 controls in winter/spring and 21 patients vs 33 controls in summer/autumn).

The other source of VitD is the dietary intake, but very few foods naturally contain an effective amount of it; therefore, diet alone is not enough to ensure adequate serum VitD levels. Certainly we can say that foods containing discretely-high amounts of VitD are unusually present in our diet (cod liver oil, salmon, mackerel, tuna). Milk and eggs contain very little amount of VitD and most foods have a VitD content of nearly null.[10] For this reason in the United States and in Canada, milk and other products such as bread, orange juices, cereals, yogurts, and cheeses are fortified with VitD,[3] and many Northern European countries fortify milk and add VitD to cereals, breads, and margarine.[23]

It is unsurprising that the dietary intake of VitD in our population of patients and controls was inferior to the recommended dose. In fact, the dietary intake of VitD was 9.7 μg/day and 12.2 μg/day in patients and controls, respectively, while the recommended intake should not be less than 10–15 μg/day. Moreover, when we matched the patients' serum levels of $25(OH)D_3$ with the corresponding intake, we found that the patients with severe deficiency take less VitD than the ones with insufficiency or normal levels (Fig. 4).

Therefore, a global nutritional strategy is required (favoring the production and use of fortified or added foods) to ensure a greater overall intake to the general population and in particular to the ones at risk.

The correction of VitD deficiency is particularly useful in patients with CHC given that low levels are associated with more severe disease progressions and reduced response to antiviral therapy.

In our study an inverse correlation between $25(OH)D_3$ and PTH levels was observed in patients and controls, suggesting that the relation is strong enough. Nonetheless, there was a significant difference in mean serum PTH levels between patients and controls ($p = 0.0004$).

A significant negative relationship between serum VitD status and PTH levels is a well known physiological event which in turn may be responsible for bone damage.

The threshold of serum $25(OH)D_3$ when serum PTH starts to increase is not universally shared, however, PTH seems to gain a nadir when $25(OH)D_3$ blood levels are between 30 and 40 ng/ml or more.[30] Based on this methodology and on the optimal efficiency of intestinal calcium absorption, the IOM report[30] and other studies[3] have defined the optimal circulating blood levels of $25(OH)D_3$ between 30 and 100 ng/ml.

In our series of patients with CHC 41/59 had VitD levels less than 30 ng/ml, but only 3 (5%) showed a PTH increase. Indeed, in the control group, a secondary hyperparathyroidism was observed in 13/59 (22%) subjects; this percentage, although more frequent than in the patient group, is lower than expected (56%).

The cause of normal or low PTH levels in presence of VitD deficiency and insufficiency observed in our studies and other research regarding chronic liver diseases[15–18] is unclear. A possible cause can be attributed to the VitD-receptor gene polymorphism (as in the case of chronic renal failure), which has been associated with relative hypoparathyroidism.[30] However, other parameters

known to interfere with PTH secretion, such as magnesium, have not been evaluated.[13,15]

In addition the mean levels of calcium and phosphate seem to be in the range of normality in both group and the difference between the population is not significant. This can be explained because of existence of other feedback mechanism.[13,15]

There are some limitations of this study. Doubtless the sample size of our study is not very high. The reason is linked to the restrictive inclusion and exclusion criteria used for the selection of patients to eliminate all confounding factors that could influence vitamin D levels. Another limitation is that the research is a case–control study drawn from a clinical series of patients and not from the community. But the cases were carefully matched or stratified by age, gender and BMI to minimize the interferences.

In conclusion, although patients with CHC are characterized by low serum levels of VitD, the deficit is comparable to that observed in the general population of the same geographical area. Consequently, we can assume that the deficit observed in patients with CHC reflects a more general problem that affects the entire population and is not limited to HCV infection.

Despite the fact that the dietary intake contributes very little to determine serum levels of VitD, patients with severe deficits are the ones that show a very low intake, about 10 times under the recommended levels. Therefore, we recommend consuming more VitD.

Finally, in patients with CHC the lack of matching increased levels of PTH, and low levels of VitD suggest a metabolic interference of VitD-PTH axis regulation.

5. 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. Moreover only the 5% of HCV patients with low levels of VitD shows a secondary hyperparathyroidism.

Author contributions

- Study concept and design: F. Morisco, N. Caporaso, A. Colao.
- Acquisition of data: F. Morisco, N. Caporaso, A. Colao, I. Loperto, F. Auriemma, M. Guarino, L. Donnarumma, V. Lembo, G. Mazzone, R. Granata, A. Mariniello, M. Rubino, F. Cariati, C. Pivonello.
- Analysis and interpretation of data: F. Morisco, N. Caporaso, A. Colao, I. Loperto, M. Guarino.
- Drafting of the manuscript: F. Morisco, N. Caporaso, A. Colao, I. Loperto, M. Guarino, C. DiSomma.
- Critical revision of the manuscript for important intellectual content: F. Morisco, N. Caporaso, A. Colao, I. Loperto, M. Guarino, C. DiSomma.
- Statistical analysis: F. Morisco, N. Caporaso, A. Colao, I. Loperto, M. Guarino.
- Study supervision: F. Morisco, N. Caporaso, A. Colao.

Conflict of interest statement

The authors declare that they have none study sponsors and none conflict of interest/financial disclosures in relation to this study.

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