Relationship between vitamin D and IL-23, IL-17 and macrophage chemoattractant protein-1 as markers of fibrosis in hepatitis C virus Egyptians

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Abstract

AIM: To assess vitamin D in hepatitis C patients and its relationship to interleukin (IL)-23, IL-17, and macrophage chemoattractant protein-1 (MCP-1).

METHODS: The study was conducted on 50 Egyptian hepatitis C virus (HCV) genotype number IV-infected patients and 25 age- and gender-matched healthy subjects. Venous blood samples were obtained. Samples were allowed to clot and sera were separated by centrifugation and stored at -20 °C. A 25 hydroxy vitamin D assay was carried out using solid phase RIA. A 1,25 dihydroxy vitamin D assay was carried out using a commercial kit purchased from Incstar Corporation. IL-17 and -23 and MCP-1 were assayed by an enzyme immunoassay. Quantitative and qualitative polymerase chain reaction for HCV virus were done by TaqMan technology. Only HCV genotype IV-infected subjects were included in the study. The mean ± SD were determined, a t-test for comparison of means of different parameters was used. Correlation analysis was done using Pearson’s correlation. Differences among different groups were determined using the Kruskal-Wallis test.

RESULTS: The mean vitamin D level in HCV patients (group I) was 15 ± 5.2 ng/mL while in control (group II) was 39.7 ± 10.8. For active vitamin D in group I as 16.6 ± 4.8 ng/mL while in group II was 41.9 ± 7.9. IL-23 was 154 ± 97.8 in group I and 6.7 ± 2.17 in group II. IL-17 was 70.7 ± 72.5 in cases and 1.2 ± 0.4 in control. MCP-1 was 1582 ± 794.4 in group I and 216.1 ± 5.38 in group II. Vitamin D deficiency affected 72% of HCV-infected patients and 0% of the control group. Vitamin D insufficiency existed in 28% of HCV-infected patients and 12% of the control group. One hundred percent of the cirrhotic patients and 40% of non cirrhotic HCV-infected patients had vitamin D deficiency. IL-23, IL-17, and MCP-1 were markedly increased in HCV-infected patients in comparison to controls. A significant negative correlation between vitamin D and IL-17 and -23 and MCP-1 was detected. HCV-infected males and females showed no differences with respect to viral load, vitamin D levels, IL-23 and MCP-1. The viral load was negatively correlated with vitamin D and active vitamin D (P = 0.0001 and P = 0.001, respectively), while positively correlated with IL-23, IL-17, and MCP-1. We classified the patients according to sonar findings into four groups. Group I a with bright hepatomegaly and included 14 patients. Group I b with perihepatic fibrosis and included 11 patients. Group I c with liver cirrhosis and included 11 patients. Group I d with hepaticellular carcinoma (HCC) and included 14 patients. Vitamin D and active vitamin D were shown to be lower in cirrhotic patients and much lower in patients with HCC, and this difference was highly significant (P = 0.0001). IL-17 and -23 and MCP-1 were higher in advanced liver disease) and the differences were highly significant (P = 0.0001).
CONCLUSION: Whether the deficiency of vitamin D is related to HCV-induced chronic liver disease or predisposing factor for higher viral load is a matter of debate.

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Key words: Vitamin D; Macrophage chemoattractant protein-1; Liver cirrhosis; Interleukin-23; Interleukin-17; Liver cirrhosis

INTRODUCTION

Vitamin D is a critical regulator of immunity, playing a role in both innate and cell-mediated immune responses. Vitamin D suppresses the production of T helper (Th)1 cytokines, such as interferon-γ (IFN-γ) and interleukin (IL)-2, and consequently leads to enhanced production of Th2 cytokines, such as IL-4 and -5, thus potentially promoting humoral immune responses. Vitamin D also promotes innate immunity by directly inducing the gene expression of antimicrobial peptides (cathelicidin and β-defensin 2) in various human cell types.[1-4]

Vitamin D deficiency has been shown to be associated with several immune-mediated diseases, and susceptibility to infection and cancer. In fact, a 25(OH)D concentration < 50 nmol/L (20 ng/mL) is an indication of vitamin D deficiency, whereas a 25(OH)D concentration of 51-74 nmol/L (21-29 ng/mL) is considered to indicate insufficiency.[5,6]

IL-23, in conjunction with IL-6 and transforming growth factor β (TGF-β), stimulates the differentiation of Th17 cells with subsequent production of IL-17.[7] IL-17 is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to IFN-γ. IL-17 acts synergistically with tumor necrosis factor (TNF) and IL-1.[8]

Chronic hepatic cirrhotic patients with genotype 1 have low 25(OH)D serum levels. Low vitamin D is linked to severe fibrosis and a low sustained virologic response (SVR) on IFN-based therapy.[9,10]

There is interesting preliminary data that indicate that 1,25(OH)2D3 suppresses Th17 driven cytokine responses, induces Treg cells, induces IL-4 production (Th2) and enhances natural killer T-cell function; differentiation and maturation of B cells is also inhibited. In addition, treatment with vitamin D receptor (VDR) agonists inhibits the T-cell production of IL-17. Furthermore, IL-17 production is sustained by IL-23, an IL-12 family member, the latter of which is strongly inhibited by VDR agonists.[11]

Also, 1,25(OH)2D3 has been shown to inhibit macrophage chemoattractant protein-1 (MCP-1)-driven inflammatory process by blocking nuclear factor-κB activation. MCP-1 is expressed in injury and inflammation and leads to direct macrophage recruitment.[12]

Hepatitis C virus (HCV) is remarkably efficient at establishing persistent infections, suggesting that HCV has evolved one or more strategies aimed at evading the host immune response. T cell responses, including IFN-γ production, are severely suppressed in patients with chronic HCV infections.[13]

Aim of the study: To assess the relationship between vitamin D and markers of inflammation in HCV infected patients and measure the degree of this relation to viral load and degree of fibrosis.

MATERIALS AND METHODS

The study approved by Ethical Committee. The study included 50 patients with HCV-related chronic liver disease with a minimum duration of 7 years (group I), who attended the Hepatology Outpatient Clinic, Endemic Disease Hospital, Faculty of Medicine, Cairo University. Collection of patients required 4 mo. Inclusion criteria were based on previous history of liver disease with HCV infection of both sexes, whether new patients or under follow up were included. Isolated HBV or coinfection with HBV and HIV infected patients were excluded.

Group I included 36 males (72%) and 14 females (28%), ranging in age from 30-65 years, with a mean age of 47.5 years. Twenty-five age- and gender-matched healthy subjects were included as a control group (group II). The controls had liver functions and abdominal U/S and test for HCV antibodies which were all normal. Informed consent was obtained from the patients and controls regarding all the procedures done.

All patients were subjected to thorough history-taking and a clinical examination. Abdominal ultrasonography was performed on all patients, and according to the results, patients were classified into 4 subgroups as follows: 14 patients with bright hepatomegaly; 11 patients with perihepatic fibrosis; 11 patients with hepatic cirrhosis; and 14 patients with hepatocellular carcinoma (HCC) and cirrhosis.

Venous blood samples were obtained after overnight fasting from all patients. Samples were allowed to clot and sera were separated by centrifugation and stored at -20 °C.

A 25 hydroxy vitamin D assay was carried out using a commercial kit purchased from Medgenix Diagnostics S.A. Zoning Industrial. B-6220 Fleurus, Belgium; Mawer,
Vitamin D, IL-23, IL-17 and MCP-1 in HCV 1980) using solid phase RIA. A 1,25 dihydroxy vitamin D assay was carried out using a commercial kit purchased from Incstar Corporation (Stillwater, MN USA; Hollis, 1986). IL-17 and -23 and MCP-1 were assayed by an enzyme immunoassay (Biosource Europe S.A). Quantitative and qualitative PCR for HCV virus were done by TaqMan technology. Only HCV genotype IV-infected subjects were included in the study.

Statistics
SPSS (version 15) was used for statistical measures of this study. The mean ± SD were determined, a t-test for comparison of means of different parameters was used. Correlation analysis was done using Pearson’s correlation. Differences among different groups were determined using the Kruskal-Wallis test.

Results
Vitamin D deficiency, defined as a serum vitamin D level < 20 ng/mL, was present in 36 patients (72%) and none (0%) of the control group. Vitamin D insufficiency (20-29 ng/mL) existed in 14 (28%), HCV-infected patients and 3 (12%) subjects in the control group. Furthermore, 25 (100%) cirrhotic patients had vitamin D deficiency and 10 (40%) non-cirrhotic HCV-infected cases. Table 1 shows the laboratory data of the study groups and demonstrates a statistically significant difference with respect to vitamin D and its active form, IL-23, and IL-17 between both groups. The viral load mean was 128 000 ± 28 000 IU/mL.

Table 1  Laboratory data of study groups

| Item                        | Group 1 (HCV infected subjects) | Group 2 (controls) |
|-----------------------------|---------------------------------|-------------------|
| 25(OH) vit D (ng/mL)        | 15 ± 5.2*                       | 39.7 ± 10.8       |
| 1,25(OH) vit D (ng/mL)      | 16.6 ± 4.8*                     | 41.9 ± 7.9        |
| IL-23 (ng/mL)               | 154 ± 97.8*                     | 6.7 ± 2.17        |
| IL-17 (ng/mL)               | 70.7 ± 72.5*                    | 1.2 ± 0.4         |
| MCP-1 (ng/mL)               | 1582 ± 794.4*                   | 216.1 ± 5.38      |

P < 0.0002. Vit: Vitamin; IL: Interleukin; HCV: Hepatitis C virus; MCP-1: Macrophage chemoattractant protein-1.

Table 2  Correlations between different parameters in hepatitis C virus infected subjects

| Items                        | R     | P value |
|------------------------------|-------|---------|
| Vit D, viral load            | -0.84 | 0.000   |
| Active D, viral load         | -0.846| 0.000   |
| Vit D and IL-23              | -0.776| 0.000   |
| Active D and IL-23           | -0.801| 0.000   |
| Vit D and IL-17              | -0.665| 0.000   |
| Active D and IL-17           | -0.679| 0.000   |
| IL-17 and viral load         | 0.951 | 0.000   |
| IL-23 and viral load         | 0.922 | 0.000   |
| MCP-1 and viral load         | 0.94  | 0.000   |
| MCP-1 and vitamin D          | -0.94 | 0.000   |
| MCP-1 and active D           | -0.92 | 0.000   |

Vit: Vitamin; IL: Interleukin; MCP-1: Macrophage chemoattractant protein-1.

Table 3  Differences between male and female subgroups of the hepatitis C virus infected patients

| Item                        | Male group (n = 27) | Female group (n = 23) | P value |
|-----------------------------|---------------------|-----------------------|---------|
| Vit D (ng/mL)               | 15.0 ± 5.12910      | 15.0 ± 5.6            | 1       |
| Active vit D (ng/mL)        | 16.6 ± 4.5          | 16.6 ± 5.3            | 0.96    |
| Viral load (IU/mL)          | 126.8 ± 96.6        | 129.3 ± 102.6         | 0.93    |
| IL-23 (ng/mL)               | 152.4 ± 96.6        | 156.1 ± 101.3         | 0.92    |
| IL-17 (ng/mL)               | 69.8 ± 69.2         | 71.9 ± 77.6           | 0.9     |
| MCP-1 (ng/mL)               | 1575.7 ± 765.4      | 1589.4 ± 844.5        | 0.9     |

Vit: Vitamin; IL: Interleukin; MCP-1: Macrophage chemoattractant protein-1.

Discussion
The liver plays a central role in vitamin D metabolism. Vitamin D inadequacy is common in non-cholestatic chronic liver diseases and correlates with disease severity. The current study showed a significant reduction of vitamin D and its active metabolites in HCV genotype 4-infected patients compared to healthy controls. This reduction was more prevalent and severe in cirrhotic vs non-cirrhotic patients. This is consistent with previous studies done on patients with genotype 1, which showed that vitamin D deficiency is universal (92%) among patients with chronic liver disease, and at least one-third of the patients have severe vitamin D deficiency[14-16].

Our results showed that IL-23 and -17 were markedly increased in HCV-infected patients in comparison to controls. Regulation of Th1 and Th17 responses in HCV-infected individuals was studied, and it was reported that TGF-β and IL-6 promote differentiation of naïve murine CD4+ T cells into IL-17-secreting Th17 cells. In addition, it has been reported that other innate cytokines, including IL-1, IL-23, TNF-α, and IL-21, in different combinations or with TGF-β, are also involved in differentiation, amplification, or stabilization of the Th17 phenotype[17,18].
Our study reported that there is a significant negative correlation between vitamin D and IL-17 and -23. Previous studies on mice showed that vitamin D is a strong inhibitor of Th17 polarization and Th17 cytokine expression of splenic CD4+ T cells. Furthermore, Th17 differentiation from naïve T cells was affected by vitamin D. These data implicate a regulatory mechanism on Th17 cells by vitamin D, through the reduction of RORgt expression\[19\].

The effect of vitamin D on the behavior of Th17 cells was investigated in different diseases and it was found that vitamin D suppressed the expression of IL-17 and -23\[20-23\].

We reported a positive correlation between IL-23 and -17 with viral load, a finding which further supports our suggestion regarding the link between vitamin D and both IL-17 and -23 in immune regulation in HCV genotype IV-related chronic liver disease. These findings may support our suggestion that increased IL-17 and -23 could, at least in part, involved in the role of vitamin D in the immune response in HCV genotype IV-related liver disease and explain how vitamin D deficiency plays a role in increasing liver fibrosis.

Our results revealed HCV-infected males and females had no differences with respect to vitamin D levels. In contrast with our results, Arte\[24]\ et al reported that African American females with chronic liver disease are at higher risk of vitamin D deficiency.

Vitamin D is an important immune modulator and preliminary data indicated an association between vitamin D deficiency and SVR rates in HCV as reduced 25-hydroxyvitamin D levels and CYPB27-1260 promoter polymorphism with reduced 1,25-dihydroxyvitamin D levels are associated with failure to achieve SVR in HCV genotypes 1-, 2-, and 3-infected patients\[9,25\]. Our HCV patients with genotype IV need further follow up to confirm the effect of vitamin D deficiency on their responses to treatment.

There was a significant increase in level of MCP-1 in our patients with all grades of hepatic affection in comparison to controls. Similar results were reported by Camps et al\[26\]. However, Panasiuk et al\[27\] reported a de-

| Item                  | Group Ia (bright hepatomegaly) (n = 14) | Group Ib (perihepatic fibrosis) (n = 11) | Group Ic (liver cirrhosis) (n = 11) | Group Id (HCC) (n = 14) | Normal (n = 25) | P value |
|-----------------------|----------------------------------------|----------------------------------------|-----------------------------------|------------------------|----------------|----------|
| IL-17 (ng/mL)         | 7.6                                    | 5.1                                    | 115.9                             | 150.3                  | 1.26           | 0.000    |
| IL-23 (ng/mL)         | 76.8                                   | 51.2                                   | 259.3                             | 225.9                  | 6.7            | 0.000    |
| Vit D (ng/mL)         | 19.8                                   | 19.4                                   | 10.9                              | 9.7                    | 39.1           | 0.000    |
| Active vit D (ng/mL)  | 20.6                                   | 21                                     | 13                                | 11.7                   | 41.1           | 0.000    |
| Viral load (IU/mL)    | 66.3                                   | 42.4                                   | 165.1                             | 231.1                  | 0              | 0.000    |
| MCP-1 (ng/mL)         | 910.3                                  | 838.8                                  | 2090.9                            | 2448                   | 237.34         | 0.000    |

Vit: Vitamin; IL: Interleukin; MCP-1: Macrophage chemoattractant protein-1; HCC: Hepatocellular carcinoma.

Table 4 Laboratory data in the four subgroups of hepatitis C virus infected subjects

Figure 1 Correlation between vitamin D (ng/mL) and interleukin-17 (ng/mL) (A), interleukin-23 (B) and viral load (C).
increase in the MCP-1 level in liver cirrhosis in comparison to the controls and did not reflect any inflammatory process in liver cirrhosis. More studies are needed to explore this point of controversy.

Our results also revealed a significant negative correlation between vitamin D and MCP-1. This supports the role of decreased vitamin D in inflammation and fibrosis. No previous work in hepatic patients studied this relationship. However, Zehnder et al. reported that reduction of the vitamin D hormonal system in kidney disease was associated with increased renal inflammation and fibrosis. Zehnder et al.[28] reported a significant negative correlation between vitamin D and MCP-1. Logistic regression analysis with urinary MCP-1 as a binary outcome showed that a 10-unit increase in serum 1,25(OH)2D or 25OHD resulted in lower renal inflammation.[28]

On classifying HCV-infected patients according to sonar finding into four groups, vitamin D and active vitamin D were shown to be lower in cirrhatic patients and much lower in patients with HCC, and this difference was highly significant ($P = 0.0001$). IL-17 and -23 and MCP-1 were higher in advanced liver disease and the differences were highly significant ($P = 0.0001$). These findings are concomitant with previous results which indicate that vitamin D inadequacy is common in non-cholestatic chronic liver diseases and correlates with disease severity.[14] The difference in viral load among these groups may explain in part the difference in levels of inflammatory cytokines.

In conclusion, vitamin D deficiency is prevalent in HCV genotype IV-infected patients and viral load is negatively correlated to vitamin D. Whether or not this deficiency is related to HCV-infected chronic liver disease or predisposing factor for higher viral load is a matter of debate. In view of the immune function of vitamin D, vitamin D status may be assessed and supplements may be considered to achieve a SVR with IFN-based therapy. The negative correlation between vitamin D and IL-23 and -17 and MCP-1 may highlight, at least in part, how these cytokines might be involved with vitamin D in immune responses in HCV genotype IV-related liver disease and may explain how vitamin D deficiency plays a role in increasing liver fibrosis.

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Innovations and breakthroughs

In view of the immune function of vitamin D, vitamin D status may be assessed and supplements may be considered to achieve a sustained virologic response with interferon-based therapy. The negative correlation between vitamin D and IL-23 and -17 and MCP-1 may highlight, at least in part, how these cytokines might be involved with vitamin D in immune responses in HCV genotype IV-related liver disease and may explain how vitamin D deficiency plays a role in increasing liver fibrosis.

Applications

IL-23, IL-17 and MCP-1 can be used as markers of degree of liver fibrosis. Vitamin D supplements may improve immune response and delays fibrosis induced by HCV.

Peer review

Authors studied the relation between serum vitamin D levels and HCV related liver disease. They detected a strong correlation with severity fibrosis, treatment response and cytokine levels which has been also shown previously.

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