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ORIGINAL ARTICLE

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 ℃. 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-17, 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 Ⅰa with bright hepatomegaly and included 14 patients. Group Ⅰb with perihepatic fibrosis and included 11 patients. Group Ⅰc with liver cirrhosis and included 11 patients. Group Ⅰd with hepatocellular 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.

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

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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)$_2$D3 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)$_2$D3 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 hepatomegly; 11 patients with perihepatic fibrosis; 11 patients with hepatic cirrhosis; and14 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 ℃.

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,

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 statistic 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 2 demonstrates the correlation between different parameters in HCV-infected subjects and controls. There was significant negative correlation between vitamin D and viral load, IL-23, IL-17 and MCP-1. Meanwhile there was a positive correlation between viral load and IL-17, IL-23 and MCP-1. Table 3 shows the studied parameters in HCV-infected patients when classified into 2 subgroups according to gender. Figure 1 show correlations between vitamin D and IL-23, IL-17, and viral load, respectively. Table 4 demonstrates the laboratory data in the four subgroups of HCV-infected 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 ($P = 0.0001$)

Table 1 Laboratory data of study groups

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

[a]$P < 0.0002$. 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
Active D, viral load	-0.846	0
Vit D and IL-23	-0.776	0
Active D and IL-23	-0.801	0
Vit D and IL-17	-0.665	0
Active D and IL-17	-0.679	0
IL-17 and viral load	0.951	0
IL-23 and viral load	0.922	0
MCP-1 and viral load	0.94	0
MCP-1 and vitamin D	-0.94	0
MCP-1 and active D	-0.92	0

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 ± 98.6	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 naive 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].

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

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
IL-23 (ng/mL)	76.8	51.2	259.3	225.9	6.7	0
Vit D (ng/mL)	19.8	19.4	10.9	9.7	39.1	0
Active vit D (ng/mL)	20.6	21	13	11.7	41.1	0
Viral load (IU/mL)	66.3	42.4	165.1	231.1	0	0
MCP-1 (ng/mL)	910.3	838.8	2090.9	2448	237.34	0

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

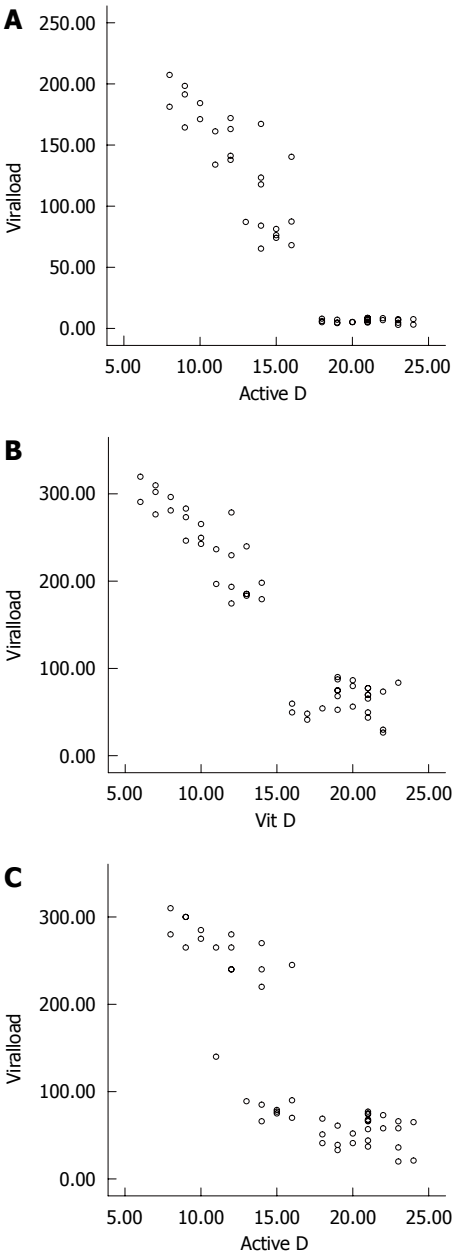

Figure 1 Correlation between vitamin D (ng/mL) and interleukin-17 (ng/mL) (A), interleukin-23 (B) and viral load (C).

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 support 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 be, 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, Arteh *et al*[24] who reported that African American females with chronic liver disease are at higher risk of vitamin D deficiency.

Our study showed that the viral load mean value was $1.28 \times 10^5 \pm 28 \times 10^3$ IU/mL. A significant negative correlation was reported between vitamin D and active vitamin D and viral load (P = 0.0001 and P = 0.001, respectively).

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 promotor 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-

crease 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*[28] 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)$_2$D 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 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). 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-induced 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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COMMENTS

Background

Vitamin D receptor (VDR) is found in significant concentrations in the T lymphocyte and macrophage populations. However, the highest concentration of VDR is in the immature immune cells of the thymus and the mature CD-8 T lymphocytes.

Research frontiers

This study highlights the relationship between interleukin (IL)-23, IL-17 and macrophage chemoattractant protein-1 (MCP-1) with vitamin D in patients with hepatitis C virus (HCV).

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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