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**Columns:** **PROSPECTIVE STUDY**

**Vitamin D status and viral response to therapy in hepatitis C infected children**

Eltayeb AA *et al*. Effect of vitamin D on viral response in children with hepatitis C

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**Abstract**

**AIM**: To study the frequency of vitamin D deficiency in patients with hepatitis C virus (HCV) infection and to evaluate the role of vitamin D supplementation in improving the antiviral therapy.

**METHODS**: About 66 children aged from 7-14 years (mean ± SD, 11.17 ± 2.293) diagnosed with HCV infection, were matched to 28 apparently healthy controls. Serum levels of 25 (OH) D3, calcium, phosphorus, alkaline phosphatase and plasma level of parathormone were measured. Quantitative PCR for HCV was done. Bone density was determined by dual energy X-ray absorptiometry. All cases received conventional therapy, and only 33 patients received vitamin D supplementation in addition.

**RESULTS**: Children with HCV showed significant increased levels of HCV RNA (*P* < 0.001), parathormone (*P* < 0.01) and decreased Vit. D level (*P* < 0.05) (33.3% deficient and 43.3% insufficient) in comparison to controls. Abnormal bone status (*Z* score -1.98 ± 0.75) was found in ribs, L-spine, pelvis and total body. Cases treated with vitamin D showed significant higher early (*P* < 0.04) and sustained (*P* < 0.05) viral response. There is a high frequency of Vitamin D deficiency among our Egyptian HCV children with significant decrease in bone density. Vitamin D level should be assessed before the start of antiviral treatment with the correction of deficiency if present.

**CONCLUSION**: Adding vitamin D to conventional Peg/RBV therapy significantly improves viral response and help in preventing the risk of emerging bone fragility.

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**Key words:** Vitamin D; Viral response; Hepatitis C; Children

**Core tip:** Does vitamin D supplementation improves the viral response in hepatitis C infection.

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**INTRODUCTION**

Hepatitis C virus (HCV) infection remains to be an evolving cause of morbidity and mortality all over the world. Despite limited epidemiological data, a higher prevalence was found in Eastern Europe and in the Middle East[1]. Egypt represents the highest prevalence of HCV infection, about 9% country wise and up to 50% in certain rural areas[2].

Vitamin D (Vit. D) is a potent immunomodulator by improving insulin sensitivity, suppressing proinflammatory cytokines, increasing anti-inflammatory cytokines, and improving CD4 T cell hyper-responsiveness[3]. Recently, specific vitamin D receptors (VDRs) is expressed in liver cells and this expression of VDRs is reduced in Chronic Hepatitis C patients. In addition, an inverse relationship between the liver VDR expression and inflammation severity has been found[4,5].

Abnormal bone metabolism, vitamin D axis, calcium (Ca) and parathormone (PTH) dysfunction have been reported in cholestatic children[6] with the disturbance in vitamin. D metabolism, gonads, or chronic inflammation[7]. The role for chronic HCV infection in osteoporosis is supported with the decreased fracture risk in HCV children with successful antiviral treatment [8]. However other studies demonstrated osteosclerosis in HCV infection[9].

The relation between vitamin D and the antiviral therapy response remains unclear with previous studies raising the possibility of inconsistent results and failure of the reference methodology[10].

To investigate the frequency of vitamin D deficiency among Egyptian HCV infected children, with the assessment of bone status by measuring calcium, parathormone, alkaline phosphatase and bone mineral density . Also to evaluate the role of vitamin D supplementation in improving the viral response of those patients.

**MATERIALS AND METHODS**

This prospective study included 66 cases (43 male and 23 female) aged from 7- 14 years with HCV infection. They were admitted in the Gastro-enterology and Hepatology unit at Assiut Children University Hospital during the period from June 2010 till December 2012. The diagnosis of HCV was based on the quantitation of HCV RNA by real-time polymerase chain reaction (PCR)[11].

***Exclusion criteria***

Patients with chronic liver disease other than HCV, patients with immunodeficiency, malignancy, decompensated liver cirrhosis, and patients on vitamin. D or calcium therapy in the previous 3 mo.

Another 28 apparently healthy volunteers children drawn from Assiut Children University Hospital of matchable age and sex were taken as control. After obtaining approval of medical ethical committee of the Faculty of Medicine, Assuit University, an informed written consent was taken from the parents or legal guardians.

**Full history taking and thorough clinical examination were performed to all the studied groups**. All cases were subjected to the following investigations: **(1) serum liver enzymes (AST and ALT), Ca, phosphorus , alkaline phosphatase, 25 (OH) D 3 and plasma parathormone (PTH) levels; (2) PCR for HCV RNA; and (3) bone mineral density (BMD)** was measured by Dual Energy X-ray Absorptiometry (DEXA) method (Hologic Model Delphi, CT, United States). Measurements of BMD (g/cm2 and Z-score) of the ribs, arms, head, lumbar spine, pelvis, legs and total body were measured. The values of BMD were compared to healthy Egyptian children matched by age- and sex. Osteoporosis was considered with a *Z*-score of −2 standard deviations (SDs) and osteopenia between −1.0 and −2.0 SDs in relation to the patient's age with reference to the World Health Organization [12].

**The patients were classified randomly by simple randomization method into 2 groups: Group A** received Peg-alpha-2b interferon (60 ug/kg per week) SC and ribavirin (15 mg/kg per day) orally for 48 wk together with vitamin D3 2000 IU/d orally**. Group B** received the same above mentioned therapy without vitamin D supplementation. Vitamin D insufficiency, deficiency and sufficiency were defined according to 25OHD levels **> 75 nmol/L,** from 75-30 nmol/L, and < 30 nmol/L respectively[13].

Follow up for all patients were done at 12 wk, 24 wk from the beginning of the therapy and 24 wk after cessation of therapy by measuring PCR for HCV RNA and liver functions. All complications and side effects during the course of treatment were recorded. 6 cases (2 from group A and 4 from group B) were excluded from the study after 12 weeks of treatment because of no responsiveness. Non-responders: Failure to clear HCV RNA from serum after 24 wk of therapy.

Discontinuation of therapy: If HCV RNA level after 12 wk < 2 log unit compared to baseline or neutropenia < 500/cm, platelets < 5000 mm³ and Hb < 8 gm/dL. Sustained virologic response (SVR) was considered when an undetectable HCV-RNA was found at 24 wk after therapy.

***Sample collection and laboratory investigations***

Venous blood samples were collected from patients under standardized conditions. Samples were centrifuged (3000 g for 10 min.), serum and plasma samples were divided and stored in aliquots at –20°C until analysis. Serum calcium, phosphorus and alkaline phosphatase were measured by conventional methods using COBAS INTEGRA 400 autoanalyser. Serum 25-OH Vitamin D measurement was done by EIA Kit from Australia Ref.K2110 basedon competition of 25 (OH) D present in the sample with 20(OH) D tracer for the binding pocket of vitamin. D binding protein. Assay of plasma levels of parathyroid hormone using MAGLUMI Intact PTH Ref.13330211001M (Sandwich immunoluminometric assay).Quantitative PCR for HCV was done using AB Applied Biosystem 7500 Fast Real-Time PCR System United States.

***Statistical analysis***

Statistical analysis was carried out by SPSS (version 16, SPSS Inc., Chicago, IL, United States). Quantitative data were expressed as mean ± SD and categorical data were presented in the form of frequency and percent (%) as appropriate. Student’s t-test was used for parametric data and non-parametric *χ*2 for independent variables in comparing the two groups. Multiple groups were compared using the one way ANOVA test. Linear correlations were performed by Pearson’s test. For all tests, the difference was considered significant if the probability (P) was < 0.05.

**RESULTS**

Positive family history of HCV was significantly different between group A and B (*P* < 0.05) (Table 1).

**The complications during the course of treatment were detected in 10 cases with mild symptoms in the form of fever, nausea, itching, headache, anaemia < 9 gm/dL, neutropenia < 5000 /cm³ or thrombocytopenia < 100.000 mm³ where the antiviral dose were reduced to half the dose.**

 Significant decrease in serum Vitamin D levels (*P* < 0.05) and significant increases in plasma PTH (*P* < 0.01) and HCV RNA (*P* < 0.001) were detected in the studied cases in comparison to the control **, despite no significant difference was found regarding liver enzymes, albumin, Phosphorus and ALP between the two groups (**Table 2)**.**

**Represents** the mean values of Bone density parameters in the studied groups with HCV infection. **There were significant differences in Z- score regarding ribs (***P* **< 0.04), pelvis (***P* **< 0.04), L-spine (***P* **< 0.05) and total BMD (***P* **< 0.03) (**Table 3)**.**

**Vit. D sufficiency was present in 23.3%, insufficiency in 43.3% and deficiency in 33.3% of cases. There were significant decrease in serum calcium levels in the deficient group *vs* the sufficient group. Serum vitamin D showed statistically significant difference between the sufficient, insufficient and deficient groups in comparison to control .The PCR for HCV RNA was significantly different in sufficient and insufficient versus the deficient groups and also in comparison to control (**Table 4)**.**

**There were significant difference in ribs (*P* < 0.04), pelvis (*P* < 0.05), L spine (*P* < 0.05) and head (*P* < 0.005) in BMD between the sufficient and deficient groups (**Table 5)**.**

**At 12 wk, there were no significance in EVR between the 2 groups, where 10 / 31 patients (32.3%) from group A and 6/ 29 (20.6%) from group B were HCV-RNA negative. At 24 wk, there were significant differences in viral response (*P* value < 0.04), where 24/ 31 patients (77.4%) from group A and 17/ 29 (58.6%) from group B had negative HCV-RNA. A significant difference in SVR was detected at 24 wk after treatment, between group A and B (*P* < 0.05). 22/ 31 patients (70.9%) from group A and 15/ 29 (51.7%) from group B were HCV-RNA negative.**

**DISCUSSION**

Egypt has the highest worldwide prevalence of HCV infection, about 9% country wise and up to 50% in rural areas[2]. This goes with our results where 15% of patients had positive family history of HCV with a significant higher percent in group A than B.

The baseline of PCR HCV RNA (> 400.000 IU/mL) was significantly higher compared to controls in this study with no significance between group A and B. Assy *et al*[13] showed that patients supplemented with vitamin. D had a significantly higher baseline of HCV RNA (60%) than those treated without vitamin D (40%). While Abu Mouch *et al*[14], found that the baseline of HCV RNA with high viral load (> 800000 IU/mL) in patients treated with vitamin D (50%) showed higher significant results than those without vitamin D treatment (42%).

In this study, vitamin **D sufficiency (> 75 nmol/mL) was present in 23.3%, insufficiency (30-75 nmol/mL) in 43.3% and deficiency (< 30 nmol/mL) in 33.3% of cases** with no significant difference between the two groups. This goes with previous authors[13,14] who found no significant difference regarding the baseline vitamin. D between cases supplemented with vitamin. D and those without. Highleyman[15] stated the importance of vitamin D in the immune response against HCV infection, as 84% of adult patients with HCV infection have low Vit. D levels and 1/3 of patients had severe deficiency.

Also in accordance, Mandorfer *et al*[16], found sufficient levels of vitamin. D in 1/5 of their participants and 57% insufficient and 23% deficient. While Ladero *et al*[17], found vitamin. D insufficiency in 40% and deficiency in 36% of adult patients.

Vitamin D status may differ across different geographic locations and environment [13]. Consequently, its influence on disease pathogenesis is likely to vary from one location to the other. vitamin. D deficiency could be related to lack of exposure to sunlight, although Africa is sunny and/or to the lack of vitamin. D in the diet[13].

Vitamin D is an important modulator of both inflammatory response and fibrosis in HCV infection by inhibiting TNF-α, that regulates the immune response, and inhibits the fibrosis process by suppressing TGF-β, which affects the fibrosis progression18. Ladero *et al*[17], concluded that Vit. D deficiency is more common in Spanish patients with HCV infection, but it is neither related to biochemical and virological varieties nor to fibrosis stage nor with IL 28B polymorphism.

On the contrary, other studies19, raises the possibility that the disease progression is associated with higher levels of vitamin D and thus vitamin D supplementation doesn't have a role in chronic hepatitis C patients. Terrier *et al*[20], showed that were no relations between serum 25 OH D3 levels and viral response to therapy or the severity of immunodeficiency in HCV patients .

This work represents significant higher plasma PTH levels in HCV patients in comparison to controls. This may be related to the functioning feedback mechanisms of low serum calcium and 25(OH)D levels. Also Malabanan *et al*[21] stated that when 25(OH)D concentration reaches < 50 nmol/L (20 ng/mL) , PTH concentration increases..

In the present study, plasma PTH levels were negatively associated with BMD and Z-score of total body. The increased PTH levels found in our patients may lead to increase bone resorption and decrease BMD. The decrease sensitivity of PTH effect on bone resorption[22] or the anabolism of the osteoblasts to PTH can be an explanation[23]. In accordance, Choudhary et al.24, found a negative correlation between PTH and BMD in viral cirrhosis. On the contrary, previous studies[25,26] revealed normal or low PTH levels in HCV infection. This discrepancy could be emphasized by the increased prevalence of 25(OH)D deficiency or insufficiency in Egyptian children with HCV infection.

In partial consistence with others[27], we observed that the peripheral skeleton were less influenced than the axial skeleton in our HCV patients. The accelerated growth in the peripheral skeleton in children could explain this while during puberty, it occurs in the axial skeleton[27]. In addition, most of our patients had been diagnosed before puberty. Therefore, the highest bone mass was obtained during the disease process which affects the bone status in these patients. The protective role of puberty on bone tends to opposite the temporary bone loss, that seems to be a complication of the disease[28].

As regarding bone density in present study, *Z* score was significantly decreased with significant difference between the two groups. This is in accordance with Elkarmouty *et al*[29], who revealed that BMD, T score and z score were significantly lower in children with HBV and HCV than HAV infection. The severity of osteoporosis increases with the severity of liver disease. However, no significant correlation could be detected between serum Ca, Phosphorus, vitamin. D and the degree of bone loss, which is more in osteoporosis than osteomalacia[29]. Kryskiewicz *et al*[6], observed some risk factors of bone tissue pathology that include; hepatocyte dysfunction, disorders of vitamin D , Ca and Phosphorus metabolism, immunosuppressive therapy and malnutrition . Theoretically disturbance in endocrine Ca - PTH- vitamin. D axis seems to play a role in the pathogenesis of osteometabolic disturbances.

López-Larramona *et al*[30], stated that the origin of hepatocyte osteodystrophy is unclear, it may be multifactorial and its etiology and severity vary in accordance with the underlying liver disease. Bone loss occurs as a result of increased bone turnover and/or remodeling in balance, being the later caused by reduced formation, and increased resorption or a combination of both. vitamin. D metabolism inturned in severe HCV infection and deficiency may cause hyperparathyroidism, increase bone turnover and accelerated loss of BMD. This regulation of RANKL/OPG system is activated by cytokines involved in the pathogenesis of chronic liver disease (IL 1, 11, 6, TNF-α1). Previous studies[24,31] found that patients with chronic liver disease have low BMD in 93% of cases. Other factors including decreased physical activity, decreased body mass, deficiency of vitamin. D and low IGF level were considered.

Yurci *et al*[32], stated that all anti osteoporotic agents in hepatic osteodystrophy seems to be safe and effective. Oral Bisphosphonate (anti resorpitive drug) was the most effective in preventing both cortical and trabecular bone loss in patients with chronic viral hepatitis, but only limited data are available.

On the contrary, Yenice *et al*[25], stated that hepatitis B and C infection do not pose a risk factor for osteoporosis and bone loss and that the diagnosis of BMD should be based on multiple parameters. It should be kept in mind that the key osteomalacia is rare and requires bone biopsy. The differences observed between reports may be related to the study design, different methods used for measuring BMD and selection of patients.

Regarding the use of vit. D in this study, cases with group A showed significantly higher percentage of EVR, higher response after 24 wk and higher SVR than group B. This agrees with previous author[14] who found that patients treated with vitamin. D in addition to conventional antiviral therapy had significantly higher response at 4 wk than those treated without vitamin. D (44% *vs* 17%), at 12 wk (94% *vs* 43%), and at 24 weeks after stoppage of therapy (86% *vs* 42%) with a small relapse rate (8%). Previous authors[13,14] concluded that the addition of vitamin. D to the therapy in HCV patients increases the rate of rapid and early SVR. Therefore assessment of vitamin. D level prior to combined therapy and correction during the course of therapy may be needed.

Beside, Bitetto *et al*[33], concluded that unfavorable responses to antiviral therapy are predicted in vitamin. D deficient patients with RHC. Supplementing vitamin. D improves the possibility to achieve a SVR after antiviral treatment. In addition, low levels of vitamin. D are associated with significant poor rate of EVR and SVR in co-infection of HIV[16] and this decreased vitamin. D is limited to fibrosis and low SVR in interferon based therapy[34]. The exact mechanism of vitamin. D supplementation action on EVR, RVR and SVR is unclear. It may be due to 1, 25 dihydroxy vitamin. D appears to be an immunomodulator via regulation of T cell function through its effect on T cell antigen[35]. T helper cell type I action is intensified when vitamin. D is insufficient or with weak VDR signals[36]. Vitamin. D increases the expression of VDR protein and inhibits the viral replication[37].

Gal-Tanamy *et al*[38], stated that the interferon sparing effect of vitamin. D improves the antiviral treatment in patients with HCV infection. 25(OH) D3 is a novel antiviral agent and a better therapeutic option in reducing the enzyme activity for patients with HCV[39].

In conclusion,there is a high frequency of Vitamin D deficiency among our Egyptian HCV children with significant decrease in bone density. Vitamin D level should be assessed before the start of antiviral treatment with the correction of deficiency if present. Adding vitamin D to conventional Peg/RBV therapy significantly improves viral response and helps in preventing the risk of emerging bone fragility.

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**COMMENTS**

***Background***

Hepatitis C virus (HCV) infection remains to be an evolving cause of morbidity and mortality all over the world. Despite limited epidemiological data, a higher prevalence was found in Eastern Europe and in the Middle East. Abnormal bone metabolism, vitamin D axis, calcium (Ca) and parathormone (PTH) dysfunction have been reported in cholestatic children.

The relation between vitamin D and the antiviral therapy response remains unclear with previous studies raising the possibility of inconsistent results

***Research frontiers***

To investigate the frequency of vitamin D deficiency among Egyptian HCV infected children, with the assessment of bone status by measuring calcium, parathormone, alkaline phosphatase and bone mineral density. Also to evaluate the role of vitamin D supplementation in improving the viral response of those patients.

***Innovations and breakthroughs***

Previous studies raises the possibility that the disease progression is associated with higher levels of vitamin D and thus vitamin D supplementation doesn't have a role in chronic hepatitis C patients. In this study cases supplemented with vitamin D showed significantly higher percentage of EVR, higher response after 24 wk and higher SVR than those without supplementation

***Applications***

To summarize the practical applications of your research findings, so that readers may understand the perspectives by which this study will affect the field and future research.

***Terminology***

The addition of vitamin D to the therapy in HCV patients increases the rate of rapid and early SVR. Assessment of Vitamin D level prior to combined therapy and correction during the course of therapy is required

***Peer review***

The study investigated the effect of vitamin D in the response to antiviral treatment in chronic infected paediatric patients with HCV. There are several works published in the literature exploring this issue, however the relation between vitamin D status and the antiviral response to therapy remains unclear.

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**Figure 1 Early and sustained viral response of hepatitis C virus RNA in group A and B.**



**Figure 2 Correlation between plasma parathormone and *Z*-score of total body.**

**Table 1 Demographic and clinical data for the studied groups and control**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **A****(*n* = 31)** | **B****(*n* = 29)** | **Total****(*n* = 60)** | **Control****(*n* = 28)** | ***P* value** |
| Age (yr) | 11.1 ± 2.178 | 11.043 ± 2.882 | 11.17 ± 2.293 | 9.35 ± 3.24 | 0.445 |
| Duration of illness (yr)  | 3.4 ± 1.984 | 3.123 ± 1.688 | 3.262 ± 1.832 | \_ | 0.563 |
| BMI (kg/m2)  | 20.81 ± 4.29 | 20.28 ± 3.13 | 20.54 ± 3.73 | 21.21 ± 2.34 | 0.588 |
|  Male/ female | 23/ 8(74.1%/25.8%) | 20/ 9(68.9%/31.1 %) | 43/17(71.6%)/ (28.3 %) | 17/13 (60.7%/46.4%) | 0.284 |
| Blood transfusion | 16(51.6 %) | 16(55.1%) | 32(53.3%) | \_ | 0.602 |
| Positive family history of HCV | 8(25.8 %) | 1(3.4%) | 9(15.0%) | \_ | 0.051 |
| Jaundice | 19(61.2%) | 13(44.8 %) | 32(53.3%) | \_ | 0.098 |
| Fever | 9(29.0 %) | 9(31.0%) | 18(30.0%) | \_ | 0.611 |
| History of bleeding | 17(54.8%) | 16(55.1 %) | 33(55.0%) | \_ | 0.501 |

Data are presented as the mean ± SD or number (%) as appropriate. 1Significance total *vs* control. No significant difference between group A and B. BMI: Body mass index.

**Table 2**  **mean ± SD values of some laboratory parameters in the studied groups and control**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Group A****(*n* = 31)** | **Group B****(*n* = 29)** | **Total****(*n* = 60)** | **Control (*n* = 28)** | ***P* value** |
| ALT (IU/L) | 75.46 ± 18.18 | 87.96 ± 14.11 | 81.71 ± 18.25 | 21.35 ± 6.21 | 0.148 |
| AST (IU/L) | 75.5 ± 24.95 | 104 ± 11.204 | 89.75 ± 23.41 | 35.74 ± 4.92 | 0.24 |
| Calcium (mg/dL) | 6.733 ± 1.52 | 6.657 ± 1.24 | 6.69 ± 1.38 | 8.2 ± 0.91 | 0.83 |
| Phosphorus (mg/dL) | 4.24 ± 0.42 | 4.21 ± 0.39 | 4.25 ± 0.41 | 3.1 ± 0.65 | 0.68 |
| Alkaline phos (IU/L) | 140.97 ± 10.98 | 99.12 ± 24.94 | 120.05 ± 27.66 | 78.48 ± 21.10 | 0.20 |
| HCV RNA (IU/mL)  | 1393,290 ± 52580 | 967371 ± 56534 | 1180334 ± 79910 | 10.21±0.74 | 0.0011 |
| Vitamin D (nmol/L) | 65.26 ± 22.71 | 57.9 ± 16.17 | 61.58 ± 17.05 | 98.31 ± 3.50 | 0.051 |
| PTH (pg/mL) | 179.10 ± 10.25 | 188.03 ± 14.96 | 186.56 ± 25.67 | 65.71 ± 12.05 | 0.011 |

1Significance total *vs* control. No significant difference between group A and B. AST: Aspartate transaminase; ALT: Alanine transaminase; PTh: Parathormone.

**Table 3 mean ± SD values of bone density parameters in the studied groups with hepatitis C virus infection**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **A****(*n* = 31)**  | **B****(*n* = 29)**  | **Total (*n* = 60)**  | ***Z*- score** | ***P* value** |
| Head  | 1.226 ± 0.394 | 1.12 ± 0.383 | 1.173 ± 0.389 | -0.16 ± 0.42 | 0.297 |
| Arm  | 1.063 ± 1.223 | 0.89 ± 0.277 | 0.976 ± 0.883 | -0.916 ± 1.2 | 0.453 |
| Leg  | 0.912 ± 0.225 | 0.969 ± 0.38 | 0.94 ± 0.311 | -1.13 ± 1.34 | 0.482 |
| Rib  | 0.688 ± 0.248 | 0.869 ± 0.411 | 0.778 ± 0.349 | -1.31 ± 0.78 | 0.0431 |
| Pelvis  | 0.839 ± 0.187 | 0.92 ± 0.238 | 0.879 ± 0.216 | -2.8 ± 1.53 | 0.051 |
| L Spine  | 0.77 ± 0.207 | 0.901 ± 0.294 | 0.835 ± 0.261 | -2.43 ± 1.7 | 0.0511 |
| Total  | 0.834 ± 0.281 | 0.913 ± 0.381 | 0.874 ± 0.334 | -1.98 ± 0.75 | 0.0361 |

1Significance between total BMD of HCV patients *vs* control. BMD: Bone mineral density (g/cm2).

**Table 4 mean ± SD values of some laboratory parameters in relation to Vitamin D status in the studied groups**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Vitamin D status** | **Control (*n* = 28)** | **Suff *vs* Insuff** | **Suff *vs* Def** | **Insuff *vs*** **Def** | **Total** ***P* value** |
| **Sufficient (*n* = 14)** | **Insufficient (*n* = 26)** | **Deficient (*n* = 20)** |
| ALT (IU/L) | 97.643 ± 18.06 | 75.654 ± 15.954 | 78.45 ± 17.824 | 21.35 ± 6.21 | 0.327 | 0.475 | 0.885 | 0.610 |
| AST (IU/L) | 97.786 ± 24.215 | 94.923 ± 13.151 | 77.4 ± 13.363 | 35.74 ± 4.92 | 0.934 | 0.453 | 0.551 | 0.772 |
| Calcium (mg/dL) | 7.993 ± 1.183 | 7.023 ± 1.396 | 6.13 ± 1.185 | 8.2 ± 0.91 | 0.769 | 0.031 | 0.09 | 0.077 |
| Phosphorus (mg/dL) | 3.31 ± 0.25 | 4.21 ± 0.31 | 4.24 ± 0.61 | 3.1 ± 0.65 | 0.354 | 0.412 | 0.614 | 0.456 |
| Alkaline phosphatase (IU/L) | 82.92 ± 15.62 | 109.34 ± 13.08 | 159.96 ± 14.64 | 78.48 ± 21.10 | 0.493 | 0.068 | 0.233 | 0.192 |
| HCV RNA (IU/mL) | 1300824 ± 664010 | 1417943 ± 137873 | 787100 ± 65915 | 10.21 ± 0.74 | 0.705 | 0.031 | 0.0221 | 0.0432 |
| Vitamin D (nmol/L) | 84.21 ± 17.821 | 49.23 ± 15.07 | 22.6 ± 3.858 | 98.31 ± 3.50 | 0.0001 | 0.001 | 0.0001 | 0.0012 |
| PTH (pg/mL) | 128.53 ± 20.37 | 164.05 ± 12.63 | 179.46 ± 19.24 | 65.71 ± 12.05 | 0.181 | 0.322 | 0.607 | 0.296 |

1Significance between groups *vs* control; 2Significance total *vs* control. AST: Aspartate transaminase, ALT: Alanine transaminase, PTh: Parathormone,

**Table 5 mean ± SD values of bone mineral density in relation to Vitamin D status in the studied groups**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Vitamin D status** | **Suff *vs* Insuff** | **Suff *vs* Def** | **Insuff *vs* Def** | **Total** ***P* value** |
| **Sufficient (*n* = 14)** | **Insufficient (*n* = 26)** | **Deficient (*n* = 20)** |
| Head  | 0.921 ± 0.343 | 1.237 ± 0.415 | 1.267 ± 0.317 | 0.020 1 | 0.005 1 | 0.790 | 0.0182 |
| Arm | 1.03 ± 0.901 | 0.879 ± 0.259 | 1.065 ± 1.325 | 0.428 | 0.931 | 0.487 | 0.759 |
| Leg | 0.873 ± 0.28 | 1.001 ± 0.321 | 0.978 ± 0.291 | 0.341 | 0.428 | 0.798 | 0.067 |
| Rib | 0.856 ± 0.34 | 0.749 ± 0.358 | 0.662 ± 0.353 | 0.365 | 0.043 1 | 0.910 | 0.637 |
| Pelvis | 0.996 ± 0.234 | 0.895 ± 0.219 | 0.748 ± 0.107 | 0.990 | 0.05 1 | 0.469 | 0.738 |
| L- Spine | 0.959 ± 0.115 | 0.838 ± 0.24 | 0.716 ± 0.258 | 0.808 | 0.0591 | 0.766 | 0.892 |
| Total | 0.918 ± 0.444 | 0.897 ± 0.11 | 0.812 ± 0.28 | 0.863 | 0.398 | 0.340 | 0.596 |
| Z score | 1.121 ± 0.577 | 0.927 ± 0.25 | -1.875 ± 0.441 | 0.619 | 0.0421 | 0.431 | 0.493 |

1Significance between groups; 2Significance total *vs* control. BMD: Bone mineral density (g/cm2).