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RESEARCH ARTICLE

Efficacy of baseline Serum 25 OH Vitamin D during enhanced Systemic inflammation in predicting response to antiviral therapy for Egyptian Patients with HCV

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Abstract

Backgrounds

A profound systemic inflammation is found in patients with chronic active HCV which could be ameliorated after antiviral therapy. Vitamin D is an important immune modulator and there is a suggested association between vitamin D and SVR.

Aims
Investigation of the effect of systemic inflammation on baseline serum 25 OH vitamin D when used as a predictor of SVR in a cohort of HCV Egyptian patients during combined antiviral therapy.

Methods
A- 378 patients were selected; 192 patients achieved SVR defined as responders group and the other is treatment failure group which included 186 patients
B- Laboratory analysis: Included routine preliminary investigations and specific investigations as quantitative detection of serum 25(OH) D using Enzyme Immuno Assay (EIA) and CRP.

Results
25(OH)D was significantly higher in the treatment failure group than the responders group and both groups showed normal level of the enzyme. In vitamin D insufficiency,CRP was significantly higher than in patients with normal level (16.2 ± 4.5 Vs. 9.87 ± 2.8 mg/L & P<0.001)

Conclusion
Serum level of 25 OH vitamin D is related to degree of hepatic steatosis but can not be used to predict SVR in case of enhanced systemic inflammatory response in HCV and it is advised to check for CRP before judging vitamin D status which if increased will make the detection of vitamin D unreliable.

Introduction

Hepatitis C virus (HCV) related liver cirrhosis is the most common indication for liver transplantation worldwide. [1] According to the World Health Organization there are 180 million people infected with the hepatitis C virus, corresponding to 3% of the world's total population.

In Egypt, a recent study found that about one person in each seven tested is positive for antibodies against HCV. However, nearly one person in ten carries the viral RNA and is therefore chronically infected. It is estimated that more than half a million people are newly infected yearly.[2]

Egypt has one of the world's highest prevalence of HCV infection, with a majority of genotype 4 infection.[3]

The current standard of care of hepatitis C treatment is combination therapy with pegylated interferon plus ribavirin for 48 weeks. This treatment is aimed to obtain a sustained virological response (SVR), which is defined as undetectable serum HCV RNA 24 weeks after termination of antiviral therapy.

Vitamin D is a steroid hormone involved in calcium homeostasis. It is present in several forms; the primary circulating form 25-hydroxyvitamin D [25(OH) D] and 1, 25 dihydroxyvitamin D [1,25(OH)2D] which is the active form.[4]

After digestion, vitamin D is exposed to 25-hydroxylases in the liver to produce 25-hydroxyvitamin D.[5] 25-hydroxyvitamin D is converted to 1,25-dihydroxyvitaminD by 25-hydroxyvitamin D-1-α-hydroxylase(CYP27B1).[6]

Serum 25(OH)D is used as a marker for vitamin D deficiency as it correlates with overall vitamin D stores. [7] The use of 1, 25(OH)2D to detect vitamin D status is doubtful due to increased biological half-life and the increased parathyroid hormone caused by vitamin D deficiency inducing renal hydroxylation of 25(OH)D via renal CYP27B1 leading to normal or elevated 1,25(OH)2D levels. [8] A circulating level of 25(OH) D below 20 ng/ml (50 nmol/l) denotes vitamin deficiency. [9]

It was postulated that the anti-viral effects of vitamin D is mediated via cathelicidin which is an antimicrobial peptide found in lysosomes in macrophages and PMNs , also through human beta defensin 2 and the release of reactive oxygen species.[10]

C-reactive protein (CRP) is the most important, sensitive and objective acute phase protein which is elevated in systemic inflammation, tissue damage, and infection. The synthesis rate of CRP is the determinant of its plasma concentration. [11]

Systemic inflammation is enhanced in patients with chronic active HCV which could be ameliorated after antiviral therapy mainly in patients who attain SVR. [12] Fat soluble vitamins including vitamin D are decreased in systemic inflammation due to altered absorption. [13]

The aim of this work is to investigate the efficacy of baseline serum 25OH vitamin D in predicting SVR in a cohort of HCV Egyptian patients exposed to combined antiviral therapy and correlation with the demographic, metabolic and histological characteristics of these patients.

METHODS

A- Patient selection

From May 2010 to November 2011, patients who were candidates for HCV antiviral therapy at the hepatology clinic-Internal medicine department-Zagazig university were followed up for one year during their course of treatment with anti viral therapy and for 6 months after termination of treatment. 378 patients were selected; patients who achieved SVR defined as responders group which included 192 patients and the other is treatment failure group which included 186 patients; 105 patients who were relapsers with reappearance of HCV RNA in serum after therapy was discontinued and 45 patients who showed viral breakthrough with reappearance of HCV RNA in serum while still on therapy, 36 patients were non-responders who fail to clear HCV RNA from serum after 24 weeks of therapy. They were enrolled after approval of the ethical committee of Zagazig university hospital. Written informed consent was obtained from patients for interview, anthropometric measurements and blood sampling. A questionnaire regarding the medical history, drug history, and family history was obtained. Patients were included if they had a histological diagnosis of chronic HCV (CHC) on a liver biopsy performed within 6 months before enrollment.

Exclusion criteria were advanced cirrhosis (Child-Pugh B and C); hepatocellular carcinoma; other causes of liver disease or mixed causes (excessive alcohol consumption, hepatitis B, autoimmune liver disease, therapy with medications known to affect vitamin D metabolism (calcium, vitamin D, bisphosphonates), previous treatment with antiviral therapy, immunosuppressive drugs.

Community based control group

This group included 30 healthy subjects after exclusion of HCV, HBV, D.M and hypertension. Alcohol consumption, therapy with medications that could affect vitamin D metabolism were additional exclusion criteria.

B- Methods

All the patients were subjected to thorough medical history taking and clinical examination. Clinical signs of portal hypertension and liver cell failure were evaluated.

C- Laboratory analysis

All patients underwent a 12-hour overnight fast

a- Routine investigations preliminary to combined therapy

- Liver function tests, prothrombin time, prothrombin concentration (%), Kidney function tests, complete Blood Count and fasting blood sugar**,** The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association, using a value of fasting blood glucose at least 126 mg/dL on at least two occasions.[14]
- HCV antibody, HBsAg, T.S.H, A.N.A, Serum AFP.
- Real time Quantitative PCR is done at 12^{th} week (COBAS Ampliprep/Taqman HCV monitor, with detection limit 15 IU/ml; Roche Diagnostic Systems
- Qualitative PCR done at 24^{th}, 48^{th} weeks of treatment and 6 months after termination of treatment using a standardized automated qualitative reverse transcription polymerase chain reaction assay (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0, with dynamic range ≥ 50 IU/ml).

-Abdominal ultrasonography

The patients were examined after 6 hours fast. Criteria of cirrhosis were excluded. Criteria of portal hypertension as Portal vein diameter more than 13mm, splenic bipolar diameter more than 130mm, splenic vein diameter>10mm**,** together with a platelet count less than 100000 with platelet count/splenic diameter ratio ≤909 [15] predict oesophageal varices and necessitates performing upper GIT endoscopy to exclude varices before therapy.

-Liver biopsy

A specimen of at least 2 cm in length was taken and fixed in 10% formalin buffer,then stained with hematoxylin-eosin to elucidate histological grading based on histological activity index (HAI) of **Knodell et al., 1981.**[16]
Staging of liver histology into F0–4 according to the Metavir scoring systems: F0 = none, F1= portal expansion, F2= bridging fibrosis, F3 = bridging fibrosis with lobular distortion, and F4 = cirrhosis. [17]
Steatosis was classified according to Kleiner Histological Scoring System. [18]

b- Specific investigations

1-Quantitative detection of serum 25(OH)D was performed using Enzyme Immuno Assay (EIA), (Immundiagnostik, Bensheim and Biomedica). Data were expressed in ng/ml. Deficiency (< 20ng/ml), insufficiency (20-30ng/ml), sufficiency (> 30ng/ml). Serum samples were withdrawn from patients prior to therapy, stored at -80 °C and tested after termination of treatment and selection of patients.

2- CRP as a marker of systemic inflammation was measured using immunoturbidimetry assay on Electa, normal level up to 5mg/L.

D- Antiviral Treatment outcome

Patients were treated with pegylated interferon 2a (Pegasys, Roche, Basel, Switzerland) 180µg /week and ribavirin at a dosage of 1000 or 1200 mg/day according to body weight for 48 weeks. Patients were withdrawn from

treatment if they did not achieve a virological response, defined as undetectable serum HCV RNA at 12, or 24 weeks after start of treatment. Sustained virological response (SVR) was defined as negative serum HCV RNA 6 months after stopping antiviral therapy.

E- Statistical Analysis

Data were analyzed using SPSS. Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequency and percentage and compared used Chi square test. The Student t test and analysis of variance were appropriately used. Spearman correlation coefficient was used to assess the relationship between vitamin D and other variables. The effects of variables on 25(OH) D were evaluated by univariate and multivariate regression analyses and to identify independent predictors of 25(OH) D serum levels.

RESULTS

The characteristics of the study patients are summarized in **Table (1)**. The cohort included 378 patient: 220 men (58%) and 158 women (42%). The mean BMI was 25.8 ± 2.5 kg/m2. Their age was 34.9 ± 8.9 years. ALT 63.1± 29.4 IU/L, AST 59.3 ± 32.3 IU/L, GGT 49 ± 23.2 IU/L , albumin 4.2 ± 0.42 g/dl , total bilirubin 1.17 ± 0.2 mg/dl, prothrombin time 11.9 ± 1.25, platelet count was 142.3 ± 43.6 $x10^3$ /µl, AFP 8.98 ± 1.4 ug/dl, FBS 105.8 ± 7.9 mg/dl, TGs 110.4 ± 42 mg/dl, C-reactive protein (CRP) 10.7 ± 3.7 mg/L, 25OH Vitamin D 40.8 ± 8.4 ng/ml. HCV RNA 420.74 ± 59.6 KIU/l.

In liver histology, 57 patients showed A3F3 (15%), 57 patients showed A1F1 (15%), 264 patients A2F2 (70%).

As regards steatosis, 180 patients had mild steatosis (47%), 99 patients (27%) had moderate degree of steatosis and only 12 patients (3%) had severe steatosis, however 87 patients (23%) had no steatosis.

The study patients were characterized into responders and treatment failure groups as shown in **Table 2**. The responders included 192 patients and treatment failure group included 186 patients. There was a highly significant statistical difference between the two groups as regards age, AST, ALT , GGT, PT, platelets count, Triglycerides, FBS, FRT, AFP, 25(OH)D (P<0.001). A significant difference was noted as regards BMI (P=0.032), albumin (P=0.049), HCV RNA (P=0.04). Non significant difference was noted in total bilirubin, CRP.

The control subjects were 16 males and 14 females with mean age of 33.4 ± 5.9 years, their mean BMI was 27.3 ± 2.3 kg/m2. All had normal ALT (24.1± 4.5 IU/L), they were not diabetic nor hypertensive; fasting blood sugar(FBS) 85.9 ± 6.1 mg/dl, triglycerides(TGs) 102 ± 15.9 mg/dl, 25OH Vitamin D level 40.3 ± 2.7 ng/ml

Serum 25 (OH) vitamin D Levels

25(OH) Vitamin D level in the study patients was 40.8 ± 8.4 ng/ml, it was higher significantly in Non-responders than controls and responders (44.3 ± 7.36, 37.43 ± 8.01 , 40.8 ± 8.4 ng/ml respectively & F=14.46, p=0.000) as shown in **Fig1**. The mean significant difference was between controls and non-responders (P=0.029) and non- responders and responders (P=0.000) as shown in **Table 3**

In responders, 25OH Vitamin D insufficiency was found in 33 patients (17%) with a mean value 25.9 ± 2.5 ng/ml, however, in treatment failure group, it is found only in 21 patients (11%) with a mean value 25.5 ± 0.92 ng/ml and that was not significant (X^2 value 0.478, P= 0.52)

CRP as a marker of systemic inflammation during antiviral therapy

In the study patients CRP was 10.7 ± 3.7 mg/L**.** In responders and treatment failure groups C-reactive protein (CRP) was 10.97 ± 3.97, 10.41± 3.44 mg/L respectively, and that was not significant (p = 0.392). In case of vitamin

D insufficiency CRP level was significantly higher than in patients with normal vitamin level (16.2 ± 4.5 Vs. 9.87 ± 2.8 mg/L & P<0.001, 95% CI: -7.88 − -4.73) as shown in **Fig 2**

Liver histology and relation to 25 OH Vitamin D level

1- Steatosis

Absence of steatosis was seen in 87 patients, mild steatosis in 180 patients, moderate steatosis in 99 patients and 12 patients showed severe steatosis. Their 25 OH vitamin D level was (43.27 ± 7.8, 39.27 ± 8.5, 41.25 ± 8.47, 39.65 ± 10.7 ng/ml respectively) and that was significant (F = 4.644, P = 0.003) as shown in **Table 4**, the least significant difference (LSD) was between (no steatosis) and (mild steatosis) subgroups. The highest level was in the subgroup with absence of steatosis (P=0.037, 95% CI: 0.24- 7.76). Mild steatosis was more frequent in hypovitaminic patients (56%) (P<0.001). The degree of steatosis was related significantly to vitamin D level.

2- Necroinflammation and fibrosis

In the study patients A2F2 was more frequent than AIFI, A3F3 [264 (70%), 57 (15%), 57 (15%) respectively, (P<0.001)]. In responders A2F2 was more frequent than A1F1, A3F3 [135 (70%), 57(30%), 0(0%) respectively, (P<0.001)]. In treatment failure patients A2F2 was more frequent than A3F3, A1F1 [129 (69%), 57 (31%), 0(0%) respectively, (P<0.001)].

As regards 25(OH)D levels according to severity of activity score, a non significant statistical relationship was found: A1F1 (40.2 ± 6.5 ng/ml), A2F2 (40.4 ± 7.3ng/ml), A3F3 (39.4 ± 6.35ng/ml) (F= 0.472, p = 0.624). A2F2 was more frequent in patients with vitamin insufficiency followed by A1F1, A3F3 (P<0.001).

Spearman rank correlation used to detect variables closely correlated to vitamin D. As candidate risk factors for serum levels of 25(OH)D, we selected age, body mass index, baseline AST, ALT, PT, platelet count, GGT, ferritin, triglycerides, fasting blood glucose, CRP, HCV-RNA levels, steatosis, and activity score. According to our result; in treatment failure group serum 25OH Vitamin D was negatively correlated with steatosis (r = - 0.287, P= 0.024) and CRP (r = - 0.263, P= 0.039) however, not correlated with degree of necro-inflammation and fibrosis (r = - 0.110, P= 0.39). In multivariate linear regression analysis, the slopes of regression lines (regression coefficients) were significantly less than zero in: Total bilirubin (β = -0.290, p=0.022, 95%CI -16.9 - -1.37) and CRP (β = -0.591, p=0.000, 95%CI -1.7 - -0.82)

In responders it is negatively correlated with CRP (r = - 0.428, P= 0.000) however, not correlated with steatosis (r = 0.141, P= 0.266) and degree of necro-inflammation and fibrosis (r = - 0.078, P= 0.54). In multivariate linear regression CRP is the only variable which can predict 25OH Vitamin D level (β = -0.474, p=0.000, 95%CI -1.41 - -0.50)

Discussion

It was suggested that Vitamin D level might play a role in SVR rates. Patients with chronic hepatitis C may be at high risk for vitamin D deficiency which may require supplementation. [19]

It was proposed that low 25(OH) D levels had been related to poor liver function because of the association between vitamin D status and hepatic function or the stage of cirrhosis. [20]

Petta et al. [21] showed that the biochemical profile of G1 CHC patients is characterized by lower than normal serum 25(OH)D levels, and that a low 25(OH)D level is related to low likelihood of SVR. Also **Kim, 2011** [22] proposed that the incidence of vitamin D deficiency is high in patients with CHC which is linked to SVR, and a CYP27B-1260 promoter polymorphism is related with poor response to antiviral combined therapy.

Bitetto et al [23] suggested that correction of vitamin D serum levels may play a complementary role to improve SVR in patients with difficult-to-treat HCV. **Abu Mouth et al** [24] showed that vitamin D level was lower in HCV patients than controls and its supplementation might improve SVR in naïve genotype 1 patients.

It was shown in our study which included a cohort of 378 Egyptian G4 CHC patients that serum level of 25OH vitamin D was normal in 324 patients (86%) 43.34 ± 0.6 ng/ml, however only 54 patients(14%) exhibited vitamin insufficiency (<30ng/ml) with a mean level of 25.7 ± 0.47 ng/ml . The vitamin level in the study patients was even slightly higher than controls with non significant statistical difference (P=0.76). Genotyping was not performed as Egypt has the highest prevalence of HCV G4, which is responsible for almost 90% of infections and is considered a major cause of chronic hepatitis. [25]

This difference in results can be attributed to conducting the study on different population with known prevalence of Genotype 4, other researches conducted on G1 mainly, also lack of data of the other studies on the potential factors that could influence vitamin D, such as exposure to sunshine, dietary intake, alcohol.

As regards relation of 25OH Vitamin D to SVR, we surprisely found that the mean level of 25OH D was significantly higher in the treatment failure group than in responders group and both groups showed normal level of the vitamin (P<0.001, 95% CI: -9.6 - -4.2), denoting that 25OH D is not a predictor of SVR .

The relation between 25OH D and acute phase response which is accentuated in chronic HCV patients and associated with decreased level of 25OH D denoted by CRP level which was significantly higher in subgroup with 25OH D insufficiency than in patients with normal level (16.2 ± 4.5 Vs. 9.87 ± 2.8 mg/L & P<0.001, 95% CI: -7.88 – -4.73) . This fact was supported by **Bonakadran and Varasteh** [26] who proposed that 25OH D deficiency was significantly correlated with inflammatory markers which contribute to CVD as CRP (p= 0.009), microalbuminuria (p=0.04), however Vitamin D not correlated with CVD.

Reid et al [27] postulated that plasma concentrations of 25(OH)D decrease after an inflammatory insult and therefore are unlikely to be a reliable measure of 25(OH)D status in subjects with evidence of a significant systemic inflammation .

As regards necro-inflammation and fibrosis, 25OH D insufficiency was observed in 54 patients of which 45 patients exhibited A2F2 in liver biopsy and only 6 patients A1F1, 3 patients A3F3 (P<0.001) denoting that Although there is a trend in 25OH D reduction with increasing stage of fibrosis, a significant reduction was also observed in the subgroup of patients with lesser degree of fibrosis, making it unlikely that low 25(OH) D levels could be entirely explained by reduced liver function. There is a non statistical difference in 25OH D among the 3 subgroups [A1F1 (40.2 ± 6.5 ng/ml), A2F2 (40.4 ± 7.3ng/ml), A3F3 (39.4 ± 6.35ng/ml) (F= 0.472, p = 0.624)].

As regards steatosis, 25OH D level was significantly different among patients with steatosis subgroups ranging from absent to severe steatosis (43.27 ± 7.8, 39.27 ± 8.5, 41.25 ± 8.47, 39.65 ± 10.7 ng/ml respectively) (F = 4.644, P = 0.003), the highest 25OH D level was seen in patients with absent steatosis 43.27 ± 7.8ng/ml. In patients with 25OH D insufficiency, mild steatosis was more frequent (56%) (P<0.001). Only steatosis was inversely correlated with 25OH D level in treatment failure group (r = - 0.287, P= 0.024).

Stauber et al [28] postulated that there is no important influence of baseline vitamin D status on responsiveness to peginterferon/ribavirin treatment in chronic HCV infection according to their findings that Vitamin D levels were not different in patients with SVR (22.0±12.1 ng/ml) vs. no SVR (22.7±14.4 ng/ml) also no significant relation was found between vitamin D levels and fibrosis stage (F0-2: 23.0±13.7 ng/ml vs. F3-4: 20.2±10.8 ng/ml).

Jazwinski [29] found no association between vitamin D levels and SVR in African American patients with HCV genotype 1. On the contrary, higher median vitamin D levels were observed in patients who failed EVR compared patients who achieved EVR.

Bellia et al [30] postulated that in extremely obese subjects, 25(OH)D serum concentrations are inversely associated with several biomarkers of systemic inflammation as CRP, IL-6, TNF-α, regardless of the total quantity of fat mass.

In conclusion , this study declared that serum level of 25OH vitamin D is related to degree of steatosis but can not be used to predict SVR in case of enhanced systemic inflammatory response in HCV. It is advised to check for CRP before judging vitamin D status which if increased will make the detection of vitamin D is unreliable.

Fig (1): Illustration of the mean of 25OH Vit. D among controls, responders and non-responders

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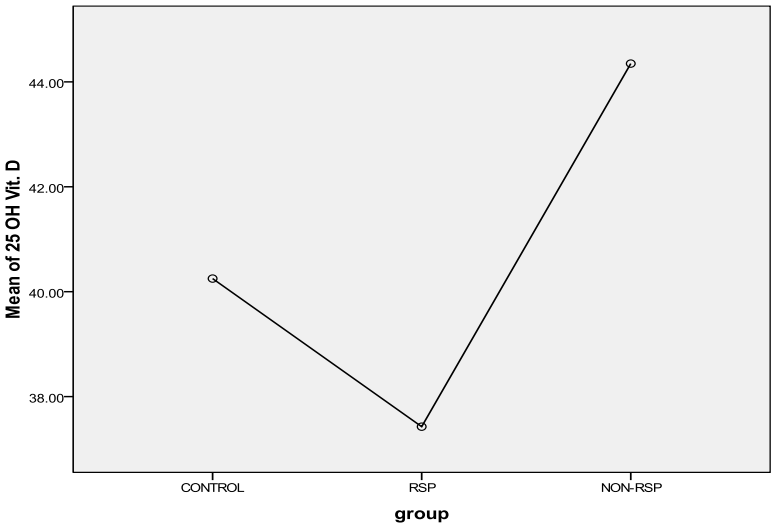

(RSP: Responders, NON-RSP: Non-responders)

Fig (2): CRP levels mg/L in normal and insufficiency states

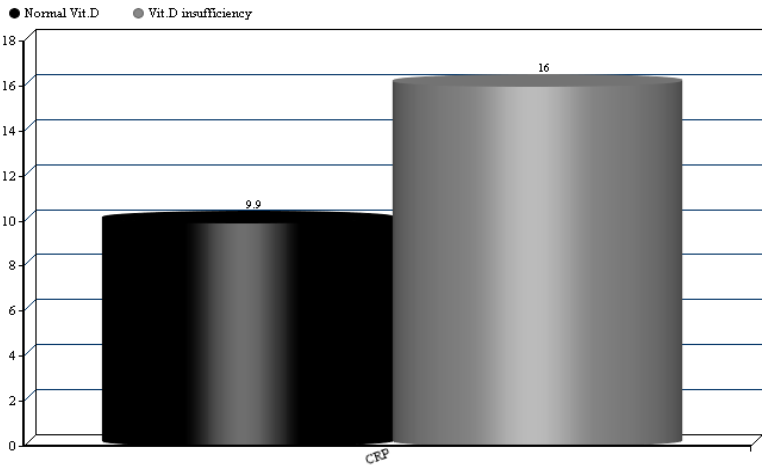

Table (1) Baseline demographic, laboratory, metabolic and Histological Features of the study Patients.

Variable	Patients (n= 378)
Age	**34.9 ± 8.9 years**
BMI	**25.8 ± 2.5 kg/m2**
ALT	**63.1± 29.4 IU/L**
AST	**59.3 ± 32.3 IU/L**
GGT	**49 ± 23.2 IU/L**
PT	**11.9 ± 1.25**
Platelet count	**142.3 ± 43.6 x103 /µl**
Albumin	**4.2 ± 0.42 g/dl**
Total Bilirubin	**1.17 ± 0.2 mg/dl**
Triglycerides(mg/dl)	**110.4 ± 42 mg/dl**
FBS (mg/dl)	**105.8 ± 7.9 mg/dl**
AFP	**8.98 ± 1.4 ug/dl**
25(OH)D (ng/dl)	**40.8 ± 8.4 ng/ml**
25(OH)D (ng/dl) <30 ng/l	
- No	**324 (86%) (**43.34 ± 0.6 ng/ml)
- Yes	**54 (14%) [33 responders, 21 Non-responders](** 25.7 ± 0.47 ng/ml)
CRP	**10.7 ± 3.7 mg/L**
CRP+ OH<30 ng/l	**16.2 ± 4.5 mg/L**
CRP+ OH>30 ng/l	**9.87 ± 2.8 mg/L**
HCV-RNA KIU/mL	**420.74 ± 59.6 KIU/l**
<u>Histology at biopsy& 25(OH)D</u>	
1- Steatosis (Kleiner classification)	
<5%	**87 (23%) (43.27 ± 7.8ng/ml)**
5% to 30%	**180 (47%) (39.27 ± 8.5 ng/ml)**
30% - 66%	**99 (27%) (41.25 ± 8.47 ng/ml)**
> 66%	**12 (3%) (39.65 ± 10.7ng/ml)**
2- necro-inflammation & fibrosis	
A1F1	**57 (15%) (40.2 ± 6.5 ng/ml)**
A2F2	**264 (70%) (40.4 ± 7.3ng/ml)**
A3F3	**57 (15%) (39.4 ± 6.35ng/ml)**

Table (2) characterization of the study patients into responders and treatment failure groups.

Variable	Responders (n= 192)	Treatment failure (n=186)	P value	95%CI
Age	30.9 ± 8.5 years	37 ± 8.2 years	<0.001	-9.1 - -3.2
BMI	25.4 ± 2.7kg/m2	26.3 ± 2.11 kg/m2	0.032	-1.8- -0.08
ALT	48.5 ± 23.7 IU/L	78.3 ± 27.2 IU/L	<0.001	-38.8- -20.8
AST	41.3 ± 10.7 IU/L	77.9 ± 36.6 IU/L	<0.001	-46.04- 27.2
GGT	31 ± 11 IU/L	67.6 ± 17 IU/L	0.001	-41.6 – 31.6
PT	11.03 ± 0.65	12.8 ± 1.1	<0.001	40 – 64.6
Platelet count	168.1 ± 31 x10^3 /µl	115.8 ± 38.6 x10^3/µl	<0.001	0.001 – 0.3
Albumin	4.3 ± 0.38 g/dl	4.13 ± 0.44 g/dl	0.049	-0.01 – 0.14
Total Bilirubin	1.2 ± 0.2 mg/dl	1.14 ± 0.23 mg/dl	0.094	-46.1- -18.6
Triglycerides(mg/dl)	94.5 ± 23.7 mg/dl	126.9 ± 50 mg/dl	<0.001	1.9 – 7.3
FBS (mg/dl)	108.1 ± 7.7 mg/dl	103.5 ± 7.5 mg/dl	0.001	1.92 - 7.29
FRT	352.6 ± 23.7	567.3 ± 37.2	<0.001	-301.4--128
AFP	3.12 ± 1.8 ug/dl	15 ± 2.7 ug/dl	<0.001	-17.2 - -6.6
CRP	10.97± 3.97 mg/L	10.41± 3.44 mg/L	0.392	-0.74- 1.88
25(OH)D (ng/dl)	37.43 ± 8.01 ng/ml	44.3 ± 7.36 ng/ml	<0.001	-9.6 - -4.2
25(OH)D (ng/dl) <30 ng/dl				
No	159(83%)(39.8±6.55ng/ml)	165(89%) (46.7 ±3.1ng/ml)		
Yes	33(17%) (25.9 ± 2.5 ng/ml)	21(11%) (25.5 ± 0.92 ng/ml)	0.52	
HCV-RNA KIU/mL	541.1 ± 113.5 KIU/l	296.5 ± 23.1 KIU/l	0.04	11.9–477.4
Histology at biopsy				
1- Steatosis (Kleiner classification)				
<5%	18 (9%)	69(37%)(15non-RSP,18 BTR, 36 RLP)		
5% to 33%	117 (61%)	21(34%)(10non-RSP, 4 BTR, 7 RLP)		
33% - 66%	57 (30%)	14(23%)(1 non-RSP, 4 BTR, 9 RLP)		
> 66%	0 (0%)	4(6%) (0 non-RSP, 0 BTR, 4 RLP)		
2-Necroinflammation & fibrosis				
A1F1	57(30%)	0 (0%)		
A2F2	135 (70%)	129 (69%)		
A3F3	0 (0%)	57 (31%)		

Table (3) LSD (least significant difference) among controls, responders and non-responders

		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CONTROL	RSP	2.82344	1.85364	.130	-.8406	6.4875
	NON-RSP	-4.09839*	1.86074	.029	-7.7765	-.4203
RSP	CONTROL	-2.82344	1.85364	.130	-6.4875	.8406
	NON-RSP	-6.92182*	1.28940	.000	-9.4706	-4.3731
NON-RSP	CONTROL	4.09839*	1.86074	.029	.4203	7.7765
	RSP	6.92182*	1.28940	.000	4.3731	9.4706

Table (4) Characterization of the study patients into 4 subgroups according to degree of steatosis

Steatosis	**No (<5%)**	**Mild (5-33%)**	**Moderate (33-66%)**	**Severe (>66%)**	**ANOVA**
No of patients	**87**	**180**	**99**	**12**	**F = 4.644**
25(OH) D	**43.27 ± 7.8**	**39.27 ± 8.5**	**41.25 ± 8.47**	**39.65 ± 10.7**	**P = 0.003**

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