IMPACT OF VIRAL ERADICATION ON HYPOVITAMINOSIS-D IN CHRONIC HEPATITIS C PATIENTS

Yasser A Abdelghani[1], Hamdy A Moukaareb[2], Mostafa Elsayed[3], Basma Magdy[4]

Article History: Received: 25.10.2020 **Revised:** 05.12.2020 **Accepted:** 15.12.2020

ABSTRACT:

Objective: We planned to explore the impact of hepatitis c virus (HCV) eradication, utilizing a sofosbuvir-based regimen with respect to level of vitamin D, grade of fibrosis.
Design: Prospective study
Setting: Minia University Hospital, Egypt
Subjects: One hundred and twenty patients with CHC were enlisted during this research. Patients were treated by sofosbuvir and ledipasvir for 12 weeks. We classified chronic hepatitis c (CHC) patients into 2 groups according to serum vitamin D level. Group 1 (Insufficient) had serum vitamin D > 20ng/ml, and group 2 (Deficient) had serum vitamin D 12- 20ng/ml. Group 3 is the control group.
Intervention: FibroScan
Main outcome measure: Serum level of vitamin D and grade of fibrosis were estimated before and after HCV treatment.
Results: Vitamin D was deficient (15.50±2.42 ng/ml) in 30% of CHC patients. The remaining 70% of CHC patients showed insufficient vitamin D level more than healthy controls (28.88±8.55 vs. 50.8±14.18 ng/ml). The grade of fibrosis was higher in CHC patients with vitamin D deficiency than CHC patients with vitamin D insufficiency (means ±SD were 10.800 ± 850.22 vs. 8.756 ± 450 pg. / ml respectively). After HCV eradication, we found a significant rise of vitamin D serum level with no change in the grades of fibrosis.
Conclusion: Clearing of HCV infection had a considerable influence on vitamin D levels but had no effect on the grade of fibrosis.
KEY WORDS: HCV treatment, vitamin D.

1 MD, Tropical Medicine and Gastroenterology, Minia University, Minia, Egypt
2 Assistant Professor, Tropical Medicine and Gastroenterology, Minia University, Minia, Egypt
3 Assistant Professor, Clinical Pathology Department, Minia University, Minia, Egypt
4 Resident of Tropical Medicine and Gastroenterology, Minia University, Minia, Egypt
Corresponding Author :Yasser A Abdelghani Email: yasser_git1@yahoo.com

INTRODUCTION:

Direct acting antiviral drugs will change the shape of the future of liver diseases, particularly in countries wherever hepatitis C infection (HCV) is the fundamental driver of liver pathology.[1] The Egyptian Demographic Health Survey's (EDHS) evaluation of HCV prevalence in persons between 15 and 59 years of age was 14.7%. Similarly, Egypt has the most elevated predominance of hepatitis C infection (HCV) in the world. Given the high prevalence of HCV among more established ages, Egypt obviously has the most noteworthy weight of cutting-edge liver disease from HCV all around. [2] As vitamin D is hydroxylated in the liver to become 25-hydroxyvitamin D, the liver is essential in its metabolism. It is then transported to the kidneys where it undergoes hydroxylation again to produce 1, 25(OH) D. [3] 25-hydroxy vitamin D can likewise change over in other cells, such as macrophages and dendritic cells, rather than the kidney cells. Liver cells, beside parathyroid organs and kidneys, express a calcium-detecting receptor that assumes a basic part in managing fundamental calcium homeostasis.[4] Derivatives of vitamin D may take part in cell proliferation, cell differentiation, and immunomodulation.[5] Vitamin D also suppresses the formation of IFNc and IL-17 for IL-10 and IL-4, which are also produced by inflammatory T cell responses.[6] Liver disorders inhibit the synthesis of vitamin D's active metabolites, which results in improper calcium and bone metabolism.[7] Patients with CHC have decreased 25(OH) D levels. Because of vitamin D's anti-inflammatory effects**,** its deficiency exacerbates chronic inflammation in HCV-positive patients. Vitamin D deficiency in chronic liver disease (CLD) patients will result in advancement of hepatic fibrosis.[8] Vitamin D receptors work out hepatocytes and hepatic stellate cells restraining stellate cell proliferation. [9,10] HSC activation is prevented by vitamin D receptor (VDR) ligands, which additionally stop liver fibrosis. Our study's objectives were to assess the prevalence of vitamin D deficiency or insufficiency within chronic hepatitis C (CHC) individuals and how it's related to

the degree of fibrosis, as well as to look into the impact of HCV elimination with a sofosbuvir-based regimen on vitamin D levels and fibrosis grade.

SUBJECTS AND METHODS:

Our study is a prospective open-label randomized study that was undertaken at Tropical Medicine Department of Minia University Hospital, Minia, Egypt, from January 2017 to December 2017. Each participant consented to participate in the trial. The study protocol was authorized by the Institutional Research Board of the Faculty of Medicine, Minia University, Egypt. The study was carried out in line with the guidelines of 1975 Helsinki Declaration. Inclusion criteria included: people >18 years old, with chronic hepatic C infection, who were treated naïve. Patient exclusion criteria included liver cirrhosis, chronic renal insufficiency (creatinine levels >1.5 mg/dL), advanced age, malabsorption syndrome, neck surgery or external radiation to the neck, alcoholism and malignancies. At the end of December 2017, the database included 127 patients with HCV. Seven patients were excluded from analysis due to incomplete follow-up data. As a control group (Group 3), 30 healthy individuals of identical age and gender were enrolled in the study. Demographic data (e.g. age, gender, and residence) were recorded. Physical examinations and laboratory tests were administered to all participants. Complete blood count was done using an automated cell counter, the Sysmex KX-21N (TAO Medical Incorporation, Japan), and kidney and liver function assessments using the auto-analyzer Konelab i60 (Thermo-electro, Clinical chemistry automation systems, Finland). The STAGO COMPACT CT Coagulation Analyzer (Diamond Diagnostics, USA) was used to test INR, and the fully automated ChemiLuminescence technology (Cobas E 411-Roche-Roche Diagnostics GmbH Germany) was used to measure HBsAg, HCV Abs, and HIV Abs. In addition, PCR for HCV RNA and serum 25-OH vitamin D3 were done to all participants. FibroScan were done for CHC patients. We classified CHC patients into 2 groups according to serum vitamin D level. Group 1 had serum vitamin D > 20ng/ml, and group 2 had serum vitamin D 12- 20ng/ml. None of the CHC patients had serum vitamin D<12 ng/ml. FibroScan grades of liver fibrosis were (F0-F1, F2, F3) and its cut-off values were 7.1 kPa for F ≥ 2, 9.5 kPa for F ≥3 and 12.5 kPa for F = 4. Vitamin D levels were assayed by EIA method (DIAsource ImmunoAssays Louvain-la Neuve, Belgium). Imaging by abdominal ultrasound and plain X-ray of lumbosacral spine was done. For a duration of 12 weeks, CHC participants who had HCV received treatment with sofosbuvir 400 mg and ledipasvir 90 mg as direct acting antivirals (DAAs). We checked the effect of HCV treatment by quantitative (q) real time (RT)-PCR in the fourth week and at the end of treatment. Plasma HCV RNA was analyzed by using the DNA technology (Moscow, Russia). Serum 25-OH vitamin D3 and FibroScan were detected in CHC patients at the end of treatment. The Ethical Research Board of the faculty of Medicine at Minia University authorized the study protocol, and the study was done in line with the 1975 Declaration of Helsinki's guidelines. Adherence to treatment was assessed on each visit based on tablet count.

Statistical analysis:

Using IBM SPSS Statistics Software (Version 21), statistical analysis was carried out. While categorical variables were summed up by percent and frequency, quantitative data were defined by the mean as a measurement of central tendency and the standard deviation as a measurement of dispersion. The significant differences in mean quantitative variables for the two patient groups were evaluated using the Student's t-test, and the significant correlation between each of the categorical variables was evaluated by Chi-square test. ANOVA was used to detect the differences between more than 2 groups. p-value was assumed to be significant if less than the threshold of 0.05 and confidence intervals were at 95% level.

RESULTS:

Our study indicated that there was no statistically significant difference in age between the three groups (Mean ±SD were 44.9±10.09, 43±10.4 and 42.9±11 years). Most participants of group 1, group 2 and group 3 were males (66.6%, 60.7%, and 60%) respectively. Both group 1 and group 2 had a more significant increase of alanine transaminase (ALT) than the control group, while group1 had a significantly lower level than group 2 (Mean ±SD were 37.6±24.23, 47.8±28.2 and 22.3±13.8U/ml), with p-value 0.004. Also, AST showed a significant difference between groups (34.8±14.97, 42.4±22 and 24.8±10.8 U/ml) in groups 1, 2 and 3 respectively with p-value 0.001. There were no significant differences in INR, total bilirubin level, albumin level, HB, platelet or total leukocytic count between the 3 groups (Table 1).

Table 2 shows that the mean serum vitamin D level in the control group (group 3) was 50.8±14.18 ng/ml. Eighty-four (70%) of CHC patients (group 1) had mean serum vitamin D level 28.88±8.55 ng/ml, significantly lower than the control group. Vitamin D was deficient (15.50±2.42 ng/ml) in 30% of CHC patients (group 2).

Although vitamin D levels were significantly lower in group 1 and 2 than group 3, bone density did not decrease in group 1 or group 2, and there was no Kyphosis (Table 3).

Table 4 shows the grades of fibrosis in CHC patients before treatment. Group 1 had 51 patients (60.70%) with grade F0-F1, 25 patients (29.80%) with grade F2 and 8 patients (9.50%) with grade F3, while group 1 had 1 patient (2.80%) with grade F0-F1, 20 patients (55.6%) with grade F2 and 15 (41.60%)

patients with grade F3. There was a significant difference in the grade of fibrosis between groups 1 and 2 with p-value 0.001.

Table 5 shows that, after treatment with sofosbuvir 400 mg plus ledipasvir 90 mg for a period of 12 weeks, AST and ALT showed significant decrease in groups 1 and 2 with p-value (0.013 & 0.014). Also, Hb and total leukocytic count showed significant decrease in groups 1 and 2 after treatment with p-value (0.023 & 0.001). No significant differences were detected in total bilirubin, albumin and platelets in groups 1 and 2 after treatment.

There was a rise in serum vitamin D level in group 1 after treatment, which was statistically significant (means ±SD were 32.99±7.43 vs. 28.88±8.55 ng/ml with p-value 0.001). In group 2, however, the level was 25.66±4.88 ng/ml after treatment vs. 15.50±2.42 ng/ml before treatment with significant differences (p-value 0.001). We did not detect any significant changes in the grades of fibrosis before or after treatment in both groups 1 and 2 (p-values 0.958 and 0.888 respectively). (Table 6)

TABLES:

Table 1: Demographic and laboratory data of the studied groups before HCV treatment

Variable	**Group *1*** **N=84**	**Group *2*** **N=36**	**Group *3*** **N=30**	**P-value**
Age (Mean ± SD)	44.9±10.09	43±10.4	42.9±11	0.522
Sex Male Female	 24(66.6%) 16(33.4%)	 51(60.7%) 33(39.3%)	 18(60%) 12(40%)	0.995
ALT Mean ± SD (U/ml)	37.6±24.23	47.8±28.2	22.3±13.8	0.0004
AST Mean ± SD (U/ml)	34.8±14.97	42.4±22	24.8±10.8	0.0001
T. Bilirubin Mean ± SD (mg/dl)	0.78±0.25	0.9±0.2	0.6±0.30	0.9684
Albumin Mean ± SD (gm./dl)	4.3±0.41	4.4±0.5	4.5±0.2	0.0475
INR Mean ± SD	1±0.1	0.9±0.3	1.1±0	0.023
Hb (gm/dl) Mean ± SD	13.1 3±1.11	12.65±1.3	13.2±1.4	0.101
TLC(x10^3 cells/cmm3) Mean ± SD	6.68±1.45	6.5±1.2	7.8±1.6	0.1013
Platelets (x10^{3}cells/cmm3) Mean ± SD	270.4±84.55	260±90.45	300.4±90..65	0.1013

Table 2: Comparison of Vitamin D level in the three groups before treatment

Group	**Number of cases**	**Mean ±SD ng/ml**
Group 1	84(70%)	28.88±8.55
Group 2	36(30%)	15.50±2.42
Group 3 (control)	30(100%)	50.8±14.18

Table 3: Effect of vitamin D on bone mineral density

Spine x ray	Group1	Group *2*	Group *3*
Spine x ray BD			
Normal	84(100%)	36(100%)	30(100%)
Decrease bone density	0(0%)	0(0%)	0(0%)
Spine x ray K			
Normal	70(100%)	30(100%)	30(100%)
Kyphosis	0(0%)	0(0%)	0(0%)

BD; bone density.K; Kyphosis.

Table 4: Grades of fibrosis in CHC patients before treatment

Grade of fibrosis	**Group1 N=84**	**Group2 N=36**	**P value**
F (0-1) (N)	51(60.70%)	1(2.8%)	< 0.001
F 2 (N)	25(29.80%)	20(55.6%)	
F 3 (N)	8(9.50%)	15(41.6%)	

N: number of patients, F: grade of fibrosis

Table 5: Demographic characteristic and laboratory findings of CHC patients before and after treatment

Variable	Group 1 **N=84**			Group 2 **N=36**		
	Before treatment	**After treatment**	**P-value**	**Before treatment**	**After treatment**	**P-value**
ALT Mean ± SD (U/ml)	37.6±24.23	30.7±9.31	0.212	45.8±28.2	32.9±8.2	0.013
AST Mean ± SD (U/ml)	34.8±14.97	29.8±6.42	0.327	42.4±22	28.10±4.5	0.014
T. Bilirubin Mean ± SD (mg/dl)	0.78±0.25	0.78±0.19	0.891	0.9±0.2	0.85±0.2	0.293
Albumin Mean ± SD **(gm/dl)**	4.3±0.41	4.38±0.42	0.491	4.4±0.5	4.5±0.4	0.138
INR Mean ± SD	1±0	1±0	1	0.9±0.2	1±0.4	0.184
HB Mean ± SD (gm/dl)	13.13±1.11	10.32±1.01	<0.001	12.10±0.3	10.10±1.2	0.023
TLC($x10^3$) Mean ±SD **(cells/cmm3)**	6.68±1.45	5.23±1.17	<0.001	6.5±1.2	5.5±1.3	0.001
Platelets($x10^3$) Mean±SD **(cells/cmm3)**	270.4±84.55	248.6±68.43	0.063	260±90.45	250.57±85.03	0.650

Table 6: Vitamin D level and grades of fibrosis after HCV treatment

Variable	Group1 N=84			Group 2 **N=36**		
	Before treatment	After treatment	P-value	before treatment	after treatment	P-value
Vitamin D Mean ±SD (ng/ml)	28.88±8.55	32.99±7.43	0.001	15.50±2.42	25.66±4.88	0.001
F(0-1) F 2 (F 3	51(60.71%) 25(29.79%) 8(9.50%)	51(60.71%) 26(30.95%) 7(8.34%)	0.958	1(2.8%) 20(55.6) 15(41.6%)	1(2.8%) 22(61.1) 13(36.1%)	0.888

DISCUSSION:

Hepatitis C infection (HCV) is viewed as one of the main reasons for liver damage around the world, ranging from liver injury and cirrhosis to hepatocellular carcinoma (HCC).[11] A decline in vitamin D 25 is an impression of decreased liver capacity or malnutrition. Another issue is that, in liver disease**,** bile creation is hampered, prompting a reduction in fat absorption and resulting in unusual take-up of vitamin D.[12] It is conceivable that HCV decreases 25(OH) D levels by changing lipid metabolism. HCV decreases creation of 7-dehydrocholesterol, the antecedent of endogenously-delivered vitamin D.[13] Because the vitamin D receptor's (VDR) transcriptional activity was reduced in the lack of vitamin D, insulin-induced gene-2 (Insig-2) expression was downregulated, and this had an inhibitory effect on the activation of sterol regulatory element-binding protein 2 and 3-hydroxy-3-methylglutaryl-coenzyme A reductase expression was appropriately elevated. So in hypovitamnosis D**,** there is an associated increase in serum cholesterol level.[14] The liver's response to injury is modulated by the vitamin D receptor (VDR), which also serves as an endocrine checkpoint. VDR ligands may be used to treat liver fibrosis. The synthetic VDR agonist calcipotriol can be used to treat liver fibrosis as it decreases the fibrotic gene expression and the collagen deposition.[10] Up to this point, restorative destruction of chronic HCV infection needs the employment of injectable IFN that are related to a major range of facet effects and suboptimal effectiveness. Most recently, mixes of direct acting antivirals (DAA) have modified the scene of hepatitis C treatment. While these spoke to a noteworthy achievement due to the high viability and ideal security profile, there is as yet a need to gather additional data about the impact of these DAA on patients after viral cure.[15] This study was undertaken at the Tropical Medicine Department of Minia University Hospital, Minia, Egypt, from January 2017 to December 2017. We intended to evaluate the prevalence of vitamin D deficiency or insufficiency among chronic hepatitis C (CHC) patients and to explore the impact of HCV eradication, utilizing a sofosbuvir-based regimen with respect to the level of vitamin D and grade of fibrosis. One hundred and twenty CHC patients were enrolled within the study along with 30 healthy controls. Our study patients were treated with sofosbuvir at 400 mg daily and ledipasvir at 60 mg daily for 12 weeks treatment. Our results indicated that, before HCV treatment**,** there were significantly lower levels of vitamin D level in chronic hepatitis patients than in the controls. We reported, in this study, that vitamin D deficiency was in 30% of CHC patients, while the remaining 70% CHC patients showed vitamin D insufficiency. These outcomes are in harmony with Petta *et al* (2010), who detailed that mean levels of serum 25(OH) D in CHC patients were significantly lower than age, and sex-coordinated controls (25.1 ± 9.9 ng/ml versus 43.1 ± 10.2 ng/ml; p <0.0001).[16] Our study has indicated that group 2 had a statistically significant increase in liver enzymes than group 1 for ALT and AST. Our findings match with those of Bahreynian et al. (2018), who observed that those with greater levels of liver enzymes had greater rates of Vitamin D shortage.[17] Our results also agree with Liangpunsakul *et al* (2011) , who reported that, in adult participants with elevation in serum, alanine aminotransferase (ALT) levels had lower levels of 25(OH)D than those with normal levels of ALT (24.7 ± 10.4 ng/mL vs 26.8 ± 10.9 ng/mL, P < 0.01).[18] Also, our results agree with Barchetta *et al* (2017), who reported different studies showing that hypovitamosis D is associated with the presence of NAFLD and steatohepatitis plus elevated transaminases level because Vitamin D reduces the expression of collagen, tissue inhibitors of metalloproteinase-1 and α-smooth muscle actin, and prevents hepatic expression of pro-fibrotic factors including the transforming growth factor (TGF) and the platelet-derived growth factor (PDGF). [19] The seriousness of vitamin D inadequacy relates to the seriousness of liver disease.[20] Vitamin D has anti-inflammatory effect, so its deficiency allows the proinflammatory cytokines and chemokines advance HCV liver disease.[21] In addition, after

HCV treatment, we found a significant rise of mean vitamin D in group 2 and group 1. All of the included CHC patients are cleared from HCV as documented by polymerase chain reaction at end of the treatment and 12 weeks afterwards. Thus, clearing of HCV infection helped to raise the level of vitamin D. The principal question is to know the mechanism that could clarify vitamin D insufficiency in HCV patients who do not have liver cirrhosis. The active HCV infection that affects the regulatory mechanisms of vitamin D synthesis may decreases 25 (OH) D levels by changing lipid metabolism. In the setting of DAA-based antiviral**,** HCV-RNA will not be detected for a number of days or weeks after the start of treatment. This is continually joined by a diminishment inflammation signals (i.e. normalization of transaminases) followed by normalization of vitamin D level.

In our study, the grades of fibrosis before HCV treatment were significantly higher in the CHC patients with vitamin D deficiency than CHC patients with vitamin D insufficiency where most of group 2 patients had F2&F3 while most of group1 patients where F0-1. According to Lange et al. (2012), fibrosis of advanced stage F2-F4 VS F0-F1 is related to low 25(OH) D, and our findings support their findings.[22] Additionally, our findings support the findings of Guzman-Fulgencio et al. (2014) that low 25(OH)D deficiencies are related to fibrosis of advanced stage (F3/4 VS F0-2)[23] but disagree with Esmat *et al* (2015), who found that no association between levels of vitamin D and stage of liver fibrosis was discovered.[24] After treatment of CHC patients**,** there was no significant change in the grades of fibrosis neither in group 1 nor in group2. Indeed, we were in need for repetition of FibroScan to our patients for a longer period of time after the SVR to confirm whether the grades of fibrosis improved after HCV treatment or not.

CONCLUSION:

Vitamin D deficiency is present in 30% of chronic hepatitis C patients while 70% of chronic hepatitis C patients have vitamin D insufficiency. Clearing of HCV infection had a considerable influence on vitamin D levels but had no effect on the grade of fibrosis.

ACKNOWLEDGMENTS:

Conflict-of-interest statement: The authors hereby certify that they have no interests in conflict.

Funding: There are no grants for this research coming from any public, commercial or not-for profit agency.

Authors' contributions:

YAA gave idea of the study, participated in the study design, recruited the patients who participated in the sequence alignment of the study and submitted the manuscript; **HM** participated in the study design and edited the manuscript; ME performed the laboratory investigation; **BM** participated in the study design and recruitment of the patients, analyzed the data and edited the manuscript.

We thank the members of Tropical Medicine and Clinical Pathology Departments of El Minia University Hospital who helped us perform our study.

REFERENCES

1- Ponziani FR, Mangiola F, Binda C, Zocco MA, *et al*. Future of liver disease in the era of direct acting antivirals for the treatment of hepatitis C. World J Hepatol. 2017 March 8; 9(7): 352-367.

2- El-Zanaty F, Way A. Egypt Demographic and Health Survey 2008. Cairo: Egypt Ministry of Health, El Zanaty and Associates, and Macro International; (Online at: http://dhsprogram.com/pubs/pdf/FR220/FR220. pdf) 2009. [Google Scholar] accessed July 22, 2017

3- Konstantakis C, Tselekouni P, Kalafateli M, *et al*. Vitamin D deficiency in patients with liver cirrhosisAnnGastroenterol. 2016 Jul-Sep; 29(3): 297–306. doi: 10.20524/aog.2016.0037 PMCID: PMC4923814.

4- Fisher L And Fisher A. Vitamin D and Parathyroid Hormone in Outpatients With Noncholestatic Chronic Liver Disease CLINICAL gastroenterology and hepatology 2007;5:513–520.

5- Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev. 2005;26:662–687

6- Jeffery L, Raza K, Filer A, *et al*. 25-hydroxyvitamin D3 conversion by dendritic cells and T cells drives 1,25-dihydroxyvitamin D3 mediated anti-inflammatory CD4+ T cell responses. Ann Rheum Dis 2011;70:A45.

7- Raman M1, Milestone AN, Walters JR, *et al*. Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer. TherapAdvGastroenterol. 2011 Jan;4(1):49-62. doi: 10.1177/1756283X10377820.

8- Hamada T, Fondevila C, Busuttil RW, *et al*. Metalloproteinase-9 deficiency protects against hepatic ischemia/reperfusion injury. Hepatology. 2008;47(1):186–198.

9- Iruzubieta P, Terán Á, Crespo J, *et al*. Vitamin D deficiency in chronic liver disease. World Journal of Hepatology. 2014;6(12):901-915.doi: 10.4254/wjh.v6.i12.901PMID: 25544877.

10-Ding N, Yu RT, Subramaniam N, *et al*. A Vitamin D Receptor/SMAD Genomic Circuit Gates Hepatic Fibrotic Response. Cell. 2013 April 25; 153(3): 601–613. doi:10.1016/j.

11-Westbrook RH, Dusheiko G. Natural history of hepatitis C. Journal of HepatologyVolume 61,

Issue 1, Supplement, November 2014, Pages S58-S68
12-Nair S. Vitamin D Deficiency and Liver Disease**.** GastroenterolHepatol (N Y). 2010 Aug; 6(8): 491–493.
13-Clark PJ, Thompson AJ, Vock DM, *et al*. Hepatitis C virus selectively perturbs the distal cholesterol synthesis pathway in a genotype-specific manner. Hepatology 2012;56:49–56.
14-Li S , He Y , Lin S , *et al*. The increase of circulating cholesterol in vitamin D deficiency is linked to reduced vitamin D receptor activity via the Insig-2/SREBP-2 pathway. Molecular Nutrition & Food Research 02 Feb 2016, 60(4):798-809] DOI: 10.1002/mnfr.201500425
15-Reig M, Marino Z, Perello C, *et al*. unexpected early tumor recurrence in patients with hepatitis C virus -related hepatocellular carcinoma undergoing interferon-free therapy: a note of caution. Jnl of Hepatology;april: 2016
16-Petta S, Cammà C, Scazzone C, *et al*. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1chronic hepatitis C. Hepatology. 2010 Apr;51(4):1158-67. doi: 10.1002/hep.23489.
17-Bahreynian M, Qorbani M , Motlagh ME, *et al*. Association of serum 25-hydroxyvitamin D levels and liver enzymes in a nationally representative sample of Iranian adolescents: The childhood and adolescence surveillance and prevention of adult noncommunicable disease study.Int J Prev Med. 2018 Feb 28;9:24. doi: 10.4103/ijpvm.
18-Liangpunsakul S, Chalasani N. Serum vitamin D concentrations and unexplained elevation in ALT among US adults. Dig Dis Sci. 2011; 56:2124–2129.
19-Barchetta I, Cimini FA, Cavallo MG. Vitamin D Supplementation and Non-Alcoholic Fatty Liver Disease: Present and Future. Nutrients. 2017 Sep 14;9(9). pii: E1015. doi: 10.3390/nu9091015.
20-Baur K, Mertens JC, Schmitt J, *et al*. Combined effect of 25-OH vitamin D plasma levels and genetic vitamin D receptor (NR 1I1) variants on fibrosi progression rate in HCV patients. Liver Int 2012; 32:635–643.
21-Almerighi C, Sinistro A, Cavazza A, *et al.* 1Alpha, 25-dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in human monocytes. Cytokine 2009;45:190–197
22-Lange CM, Bibert S, Kutalik Z, *et al*. A genetic validation study reveals a role of vitamin D metabolism in the response to interferon-alfa-based therapy of chronic hepatitis C. PLoS One 2012; 7: e40159 [PMID: 22808108 DOI: 10.1371/journal.pone.0040159]
23-Guzmán-Fulgencio M, García-Álvarez M, Berenguer J, *et al*. Vitamin D deficiency is associated with severity of liver disease in HIV/HCV coinfected patients. J Infect 2014; 68: 176-184 [PMID: 24184809 DOI: 10.1016/j.jinf. 2013.10.011]
24-Esmat G, El Raziky M, Elsharkawy A, *et al*. Impact of vitamin D supplementation on sustained virological response in chronic hepatitis C genotype 4 patients treated by pegylated interferon/
ribavirin. J Interferon Cytokine Res 2015; 35: 49-54 [PMID: 25061714 DOI: 10.1089/jir.2014.0060]