Association between Serum Vitamin D, Insulin Resistance and Severity of Liver Fibrosis in Chronic Hepatitis C Patients

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ABSTRACT--- ***Liver Fibrosis is a worldwide problem; its diagnosis depends on different modalities as Acoustic Radiation Force Impulse Imaging. Recently, Vitamin D and insulin resistance were linked to liver fibrosis. We aimed to study the association between serum Vitamin D, insulin resistance measured by HOMA-IR and liver fibrosis assessed by Acoustic Radiation Force Impulse Imaging in chronic Hepatitis C patients. The study carried on 60 male patients with chronic Hepatitis C (Group I), and 20 age and sex matched healthy subjects (Group II). All subjects included in this study were subjected to: history taking, routine laboratory investigations, fasting plasma insulin and fasting plasma glucose levels to calculate HOMA-IR, serum Vitamin D level as well as Acoustic Radiation Force Impulse Imaging for assessment of liver fibrosis. HOMA-IR and Acoustic Radiation Force Impulse Imaging readings were significantly high in chronic Hepatitis C patients in comparison to normal controls. On the other hand, serum Vitamin D levels were evidently low among Hepatitis C patients in comparison to normal controls. Acoustic Radiation Force Impulse Imaging, HOMA-IR and serum Vitamin D levels have high ability for the diagnosis of liver fibrosis in Hepatitis C patients.***

Keywords--- Vitamin D, Insulin resistance, HOMA-IR, Liver fibrosis, Acoustic Radiation Force Impulse Imaging, Hepatitis C Virus

INTRODUCTION

Since its discovery in 1989, hepatitis C virus (HCV) has been recognized as a major cause of chronic liver disease and liver fibrosis worldwide. The estimated global prevalence of HCV infection is 2.2%, corresponding to about 130 million HCV-positive persons worldwide. (Bode JG et al, 2007, 254-65) The lowest prevalence (0.01%-0.1%) has been reported from countries as the United Kingdom and Scandinavia, while the highest prevalence (14.7%) has been reported from Egypt according to the Egyptian Demographic Health survey (EDHS). (Frank C et al, 2000, 887-91) Today, HCV infection and its complications are among the leading public health challenges in Egypt. (Miller FD and Abu Raddad LJ, 2010, 14757-62)

Liver fibrosis and cirrhosis in chronic HCV infection is the result of wound-healing response to injury, where liver fibrosis entails major alterations in both quantity and quality of hepatic extra cellular matrix (ECM) and there is overwhelming evidence that activated hepatic stellate cells (HSC) are the major producers of this matrix. Following chronic liver injury, HSC proliferate; lose their vitamin A contents and undergo a major phenotypical transformation to smooth muscle α-actin positive myofibroblasts (activated HSC) which produce a wide variety of collagenous and non-collagenous ECM proteins. (Friedman SL, 2003, 38–53) and (Marra F, 1999, 1120–30)

Liver biopsy was the gold standard for diagnosis of liver fibrosis; however, liver biopsy is an invasive procedure with associated morbidity, mortality and carries a significant cost per procedure. Therefore, the role of liver biopsy in patients with chronic HCV has increasingly been questioned. (Perrillo R, 1997, 57-6) Over the last few years, several imaging modalities were proposed for the diagnosis of liver fibrosis in chronic HCV patients. Acoustic Radiation Force Impulse (ARFI) Imaging is an ultrasound based diagnostic technique that evaluates tissue stiffness. ARFI is implemented in the ultrasound scanner and by using a conventional probe it evaluates deep tissues stiffness providing complementary information potentially useful for the diagnosis. (Friedrich-Rust M et al, 2009, 595–604)

Vitamin D (Vit D) is a fat soluble vitamin which can be considered both a vitamin and a hormone with known actions as calcium and phosphate homeostasis, development and maintenance of bone, together with potent antiproliferative; prodifferentiating and immunomodulatory activities. (Kochupillai N, 2008, 256-62)

Vit D itself is biologically inactive; therefore, it must be metabolized to its biologically active forms. Vit D enters the circulation carried on a specific protein and is transported to the liver. In the liver, the first step in the metabolic activation of Vit D is the hydroxylation of carbon 25 via mitochondrial cytochrome P-450 enzyme to form 25-hydroxy vitamin D [25(OH)D], the major circulating form of Vit D. In the kidneys and other tissues, the 25(OH)D-l-alpha hydroxylase enzyme catalyzes a second hydroxylation of 25(OH)D, resulting in the formation of 1 alpha 25-dihydroxyvitamin D [l,25(OH)2D] the most potent form of Vit D. (Lips P, 2006, 4-8)

Insulin resistance (IR) describes an impaired biological response to normal levels of circulating insulin. Homeostasis model assessment of insulin resistance (HOMA-IR) is used to yield an estimate of insulin sensitivity and β-cell function from fasting plasma insulin and glucose levels, where higher HOMA-IR score suggests greater degree of IR. HOMA-IR was calculated using the formula: HOMA-IR = fasting insulin (μU/ml) x fasting glucose (mg/dl)/405. (Le Roith D et al, 2001, 588-97) and (Hanley AJG et al, 2003, 463-9)

In many studies, the extent and rate of hepatic fibrosis progression were associated with IR, where IR in chronic HCV is closely linked to the hepatic inflammatory response against the virus. It is well known that chronic HCV is associated with increased level of tumor necrosis factor alpha (TNF-α), where TNF-α elevation is associated with IR through interference with insulin signaling. (Kumar D et al, 2002, 1266–72) In addition, hyperinsulinemia itself may be a key factor in hepatic fibrogenesis where hyperinsulinemia directly stimulates HSC proliferation and secretion of ECM with up regulation of connective tissue growth factor, a key profibrogenic cytokine. (Paradis V et al, 2001, 738–44)

Vit D levels are inversely associated with IR and diabetes in epidemiological studies, also, Vit D improves insulin sensitivity of the target cells (liver, skeletal muscle, and adipose tissue), even in subjects with normal glucose tolerance. The mechanisms proposed which may underlie this effect include improvement in lean mass, regulation of insulin release, altered insulin receptor expression and enhancement of β-cell function. (Takiishi T et al, 2010, 419–46)

Moreover, there is growing evidence that Vit D status is related to chronic liver disease. In chronic HCV infection, Vit D status has been inversely correlated with liver fibrosis progression; where Vit D has antiproliferative and antifibrotic effects on the liver, and may have potential therapeutic value as a preventive and/or early treatment strategy for hepatic fibrosis. (Stokes CS et al, 2013, 338-52) Thus, the Endocrine Society Clinical Practice Guideline (ESCPG) recommended screening for Vit D deficiency in individuals at risk for deficiency, including those with chronic liver disease, and Vit D supplementation for deficient patients. (Holick MF et al, 2011, 1911–30)

The aim of the present work was to study the association between serum Vit D level, IR measured by HOMA-IR and the severity of liver fibrosis assessed by ARFI imaging in chronic HCV patients. Also, to compare these values with different studied clinical and laboratory parameters.

SUBJECTS AND METHODS

The study was carried on 60 male patients with chronic HCV recruited at Internal Medicine Department, Alexandria Main University Hospital and Alexandria Fever Hospital **(Group I)**. Also, 20 age and sex matched healthy subjects were included as a control group **(Group II)**. Exclusion criteria for patients include: chronic liver diseases other than chronic HCV, previous treatment with antiviral therapy for HCV, hepatocellular carcinoma, treatment with steatosis induced drugs (as methotrexate; glucocorticoids, L-asparginase and tetracycline), treatment with medications known to affect Vit D metabolism (as antiepileptic drugs; glucocorticoids; bisphosphonates and antiretroviral drugs), any known kidney diseases and diabetes mellitus.

All subjects included in this study were subjected to the following:

- Thorough history taking stressing on symptoms and signs of chronic liver disease [as fatigue, jaundice, ascites, gastrointestinal (GIT) bleeding, lower limb edema and hepatic encephalopathy], together with complete clinical examination both general and local abdominal examination [to assess the condition of the liver, the spleen as well as detection of ascites].
- Laboratory Investigations; including:
  1-Complete blood count (CBC).
  2-Liver biochemical profile; including: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin activity, serum bilirubin and albumin levels.
  3-Viral markers; including: HCV antibodies, HBs Ag and quantitative PCR for HCV RNA in HCV positive patients.
  4-Serum Vit D [25(OH)D] level.
  5-Fasting plasma insulin and fasting plasma glucose levels to calculate HOMA-IR using the following equation:

$$\frac{Fasting\ Insulin\ \mu U/ml \times Fasting\ Glucose\ mg/dl}{405}$$

- Acoustic radiation force impulse (ARFI) imaging to assess the degree of liver fibrosis.

The study was done after approval of the Faculty of Medicine, Alexandria University Medical Ethical Committee, and an informed written consent from all the subjects enrolled in the study was taken.

RESULTS

Demographic data:

All our selected patients and control subjects were males. Regarding age, it showed close means of 55.6±6.59 years and 54.45±7.23 years in **Group I** and **Group II** respectively.

Clinical presentation of HCV patients:

Table (1) showed that fatigue and jaundice were the common presenting manifestations among our studied HCV patients (41.7%). Also, ascites; GIT bleeding; lower limb edema and hepatic encephalopathy were reported with variable percentages (38.3%, 30%, 23.3% and 18.3% respectively).

Table (1): Clinical presentation among Group I HCV patients.

Clinical presentation	Cases (n=60)	%
Fatigue	25	41.7
Jaundice	25	41.7
Ascites	23	38.3
GIT bleeding	18	30.0
Lower limb edema	14	23.3
Hepatic encephalopathy	11	18.3

Studied laboratory parameters:

CBC findings: **Table (2)**

Pancytopenia was found in HCV patients (**Group I)** in comparison to control group (**Group II)**, with evident statistical significant difference between both groups where p <0.001.

Table (2): Comparison between the two studied groups according to CBC findings.

	Cases (n= 60)	Control (n= 20)	Test of sig.	P
Hb (g/dl)				
Min. – Max.	7.40–14.40	11.0–14.0		
Mean ± SD.	10.83±2.03	12.60±0.86	t= 3.785*	<0.001*
Median	11.0	12.50		
Platelets ($\times10^3$) (cell/cmm)				
Min. – Max.	50.0–280.0	160.0–290.0		
Mean ± SD.	149.22±63.53	226.50±38.84	Z= 4.536*	<0.001*
Median	151.0	225.0		
WBC_S ($\times10^3$)				
Min. – Max.	1.62–10.0	4.0–8.0		
Mean ± SD.	4.38±2.02	5.68±1.20	Z= 3.409*	0.001*
Median	3.75	5.35		

t: Student t-test for comparing between the two groups
Z: for Mann Whitney test for comparing between the two groups *: Statistically significant at $p \leq 0.05$

Liver biochemical profile: **Table (3)**

A significant increase in the level of liver enzymes (ALT, AST) and serum bilirubin in (**Group I)** HCV patients in comparison to control subjects (**Group II)** was reported. On the other hand, a significant decrease in prothrombin

activity and serum albumin level was observed in **Group I** HCV patients in comparison to **Group II** control subjects. An evident statistical significant difference was observed between both studied groups, where p <0.001.

Table (3): Comparison between the two studied groups according to liver biochemical profile.

	Cases (n= 60)	Control (n= 20)	Test of sig.	P
ALT (IU/L)				
Min. – Max.	23.0–124.0	9.0–19.0		
Mean ± SD.	55.27±23.22	12.75±2.88	Z= 6.673*	<0.001*
Median	54.0	12.0		
AST (IU/L)				
Min. – Max.	14.0–94.0	11.0–21.0		
Mean ± SD.	54.17±19.30	16.80±3.59	t= 14.271*	<0.001*
Median	52.0	17.50		
Total bilirubin (mg/dl)				
Min. – Max.	0.40–3.60	0.60–1.0		
Mean ± SD.	1.86±0.91	0.78±0.12	t=8.991*	<0.001*
Median	1.60	0.80		
Prothrombin activity (%)				
Min. – Max.	42.0–98.0	89.0–102.0	t= 13.602*	<0.001*
Mean ± SD.	70.03±15.09	97.90±2.83		
Median	74.50	98.0		
Albumin (g/dl)				
Min. – Max.	1.70–4.30	4.20–5.10		
Mean ± SD.	3.13±0.76	4.84±0.28	t= 14.807*	<0.001*
Median	3.20	5.0		

t: Student t-test for comparing between the two groups
Z: for Mann Whitney test for comparing between the two groups *: Statistically significant at p ≤ 0.05

HOMA-IR: **Table (4)**

HOMA-IR values were high among **Group I** HCV patients in comparison to **Group II** control subjects, where the means were 3.21±0.92 and 2.26±0.36 in both groups respectively. Moreover, an evident statistical significant difference was observed between both studied groups where p <0.001.

Table (4): Comparison between the two studied groups according to HOMA-IR.

	Cases (n= 60)	Control (n= 20)	t	P
HOMA-IR				
Min. – Max.	1.51–4.99	1.6 –2.86		
Mean ± SD.	3.21±0.92	2.26±0.36	6.560*	<0.001*
Median	3.30	2.29		

t: Student t-test for comparing between the two groups *: Statistically significant at p ≤ 0.0

Vitamin D [25 (OH)D] level: **Table (5)**

A significant decrease in serum Vit D level among HCV patients (**Group I)** was reported in comparison to control group (**Group II)**, where the means were 6.63±4.26 ng/ml and24.16±8.85 ng/ml in both groups respectively. A statistical significant difference was observed between both studied groups, where p <0.001. Moreover, all our studied HCV patients had Vit D deficiency. On the other hand, 30% of the normal control subjects had Vit D deficiency, 40% had Vit D insufficiency and 30% had normal sufficient Vit D.

Table (5): Comparison between the two studied groups according to serum vitamin D (Vit D) level.

	Cases (n= 60)		Control (n= 20)		Test of sig.	p
	No.	%	No.	%		
25 (OH) D						
Min. – Max.	1.0–17.60		11.12–50.30			
Mean ± SD.	6.63±4.26		24.16±8.85		t= 8.536*	<0.001*
Median	5.55		22.44			
Deficiency (<20)	60	100.0	6	30.0		
Insufficient (20 - 29)	0	0.0	8	40.0	χ^2= 50.909*	MCp <0.001*
Sufficient (30 - 100)	0	0.0	6	30.0		

X^2, p: Chi square test for comparing between the two groups
t: Student t-test for comparing between the two groups *: Statistically significant at p ≤ 0.05

Acoustic Radiation Force Impulse (ARFI): Table (6)

ARFI readings in HCV patients (**Group I)** ranged from 1.13–2.97 m/s with a median of 1.75 m/s, these values were much higher in comparison to ARFI readings of control group (**Group II)** which ranged from 0.80–1.10 m/s with a median of 0.89 m/s. A statistical significant difference was detected between both studied groups as regards liver stiffness, where liver stiffness increased evidently in HCV patients.

Table (6): Comparison between the two studied groups according to ARFI readings.

	Cases (n= 60)	Control (n= 20)	t	P
ARFI				
Min. – Max.	1.13–2.97	0.80–1.10		
Mean ± SD.	1.80±0.45	0.92±0.10	13.987*	<0.001*
Median	1.75	0.89		

t: Student t-test for comparing between the two groups *: Statistically significant at p ≤ 0.05

Correlation between serum Vit D level/ARFI readings and different studied laboratory parameters in Group I HCV patients: Table (7)

Serum Vit D level showed positive correlation with platelets count and negative correlation with HOMA-IR. On the other hand, ARFI readings showed positive correlation with HOMA-IR and negative correlation with serum Vit D level.

Table (7): Correlation between serum Vit D level/ARFI readings and different studied laboratory parameters in Group I HCV patients.

	Vit D		ARFI	
	r	p	r	p
HCV-RNA	0.201	0.123	-0.235	0.071
ALT	-0.033	0.804	0.112	0.394
AST	-0.053	0.687	-0.111	0.399
Prothrombin Activity	0.129	0.324	0.238	0.067
T. bilirubin	-0.182	0.165	-0.104	0.430
Albumin	0.215	0.099	0.206	0.114
Platelets	0.315*	0.014*	0.067	0.613
HOMA-IR	-0.309*	0.016*	0.544*	<0.001*
Vitamin D			-0.486*	<0.001*

r: Pearson coefficient *: Statistically significant at $p \leq 0.05$

Sensitivity, specificity and accuracy of Vit D and ARFI in the diagnosis of hepatic fibrosis in HCV Group I patients:

The receiver operating characteristics (ROC) analysis to study Vit D diagnostic ability in the diagnosis of hepatic fibrosis showed that Vit D at the best cut-off value of 14.3 ng/ml had a sensitivity of 95%, specificity of 90%, positive predictive value (PPV) of 96.61 %, negative predictive value (NPV) of 85.71% and area under the curve (AUC)= 0.985.

Moreover, ROC analysis to study ARFI diagnostic ability in the diagnosis of hepatic fibrosis showed that ARFI at the best cut-off value of 1.1 m/s had a sensitivity of 100%, specificity of 100%, positive predictive value (PPV) of 100 %, negative predictive value (NPV) of 100% and area under the curve (AUC)= 1.000.

DISCUSSION

In the present study, we excluded females because there are some evidences that the serum level of Vit D differs by sex and insufficient status has been linked to female sex. (Daly RM et al, 2012, 26–35) Regarding age, it showed close means of 55.6±6.59 years and 54.45±7.23 years in **Group I** and **Group II** respectively. This agrees with the largest population-based study in Egypt, where HCV prevalence was significantly increased in the fifth decade. (Abdel-Aziz F et al, 2000, 111–5)

Fatigue; jaundice; ascites; GIT bleeding; lower limb edema and hepatic encephalopathy were reported among our studied HCV patients with variable percentages. This was consistent with other authors who reported similar presenting manifestations among their studied patients. (Poynard T et al, 2002, 295-303) and (Stephen L et al, 2006; 47–52)

As regards hematological findings, pancytopenia was found in our studied HCV patients, this finding agrees with the results of a study made by Streiff MB et al, 2002, 947-52. Also, disturbed liver biochemical profile among our studied HCV patients was in consistent with other studies. (Faisal MS et al, 2008, 1011-8) and (Croquet V et al, 2002, 1133- 41)

In the present study, HOMA-IR values were significantly higher in HCV patients **(Group I)** in comparison to control subjects **(Group II)**. This was reported by other authors who found that IR was evident among HCV patients. (Maeno T et al, 2003, 1358- 63) and (Kawaguchi T et al, 2004, 1499-508)

In the present work, there was a significant decrease in serum Vit D level among HCV patients **(Group I)** in comparison to control subjects **(Group II)**, this was in agreement with several studies which reported low serum Vit D levels among HCV and hepatic decompensation patients. (Petta S et al, 2010, 1158–67) and (Mariana CS et al, 2015, 99-107)

In our study, there was a significant difference between both studied groups regarding ARFI readings ($p < 0.001$), where ARFI readings were evidently high among HCV patients **(Group I)**. This was in accordance with many recent studies. (Castéra L et al, 2005, 343–50), (Sporea I et al, 2010, 26-31) and (Su-Mei L et al, 2014, 9528–33)

In the present study, there was an evident negative correlation between serum Vit D level and IR measured by HOMA-IR. This was in agreement with Gandhe MB et al, 2013, 2438, who found that Vit D levels were much lower in diabetic patients when compared to non-diabetics. On the other hand, ARFI readings showed positive correlation with

HOMA-IR and negative correlation with serum Vit D level. In accordance with our results, studies showed that low levels of Vit D are associated with fibrosis and suggested that these low levels may predict hepatic decompensation and mortality in patients with chronic liver failure. (Putz-Bankuti C et al, 2012, 845–51) and (Baur K et al, 2012, 635–43)

In our study, Vit D at the best cut-off value of 14.3 ng/ml and ARFI at the best cut-off value of 1.1 m/s had a high ability for the diagnosis of liver fibrosis. This was in accordance with other studies which reported nearby values. (Garcia-Alvarez M et al, 2014, 1541–50) and (El Said HI et al, 2014, 79–82)

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REFERENCES

- Abdel-Aziz F, Habib M, Mohamed MK, Abdel-Hamid M, Gamil F, Madkour S, et al. Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. Hepatology 2000; 32: 111–5.
- Baur K, Mertens JC, Schmitt J, Iwata R, Stieger B, Eloranta JJ, et al. Combined effect of 25-OH vitamin D plasma levels and genetic vitamin D receptor (NR 1I1) variants on fibrosis progression rate in HCV patients. Liver Int 2012; 32: 635–43.
- Bode JG, Brenndörfer ED, Häussinger D. Subversion of innate host antiviral strategies by the hepatitis C virus. Arch Biochem Biophys 2007; 462[2]: 254-65.
- Castéra L, Vergniol J, Foucher J. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005; 128: 343–50.
- Croquet V, Vuillemin E, Ternisien C, Pilette C, Oberti F, Gallois Y, et al. Prothrombin index is an indirect marker of severe liver Fibrosis. Eur J Gastroenteral Hepatol 2002; 14: 1133- 41.
- Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol 2012; 77: 26–35.
- El Said HI, Ashraf NE, Fathallah SM, Perihan ES, Sarah FM. Study of hepatic stiffness in hepatitis C virus-related liver diseases by acoustic radiation force impulse imaging. Egyptian Liver J 2014; 4[3]: 79–82.
- Faisal MS, Ali B, Hussa AH, Suhail A, Osama A, Ayman AA, et al. Is serum transaminase level a reliable marker of histological disease in chronic Hepatitis C infection. Liver Int 2008; 28[7]: 1011-8.
- Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, Elkhoby T, Abdel-wahab Y, Aly Ohn ES, Anwar W, Sallam I. The role of parentral antishistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000; 355: 887-91.
- Friedman SL. Liver fibrosis-from bench to bedside. J Hepatol 2003; 38: 38–53.
- Friedrich-Rust M, Wunder K, Kriener S, Sotoudeh F. Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. Radiology 2009; 252: 595–604.
- Gandhe MB, Jain K, Gandhe SM. Evaluation of 25 (OH) Vitamin D3 with Reference to Magnesium Status and Insulin Resistance in T2DM. JCDR 2013; 7[11]: 2438.
- García-Álvarez M, Pineda-Tenor D, Jiménez-Sousa MA, Fernández-Rodríguez A, Guzmán-Fulgencio M, Resino S. Relationship of vitamin D status with advanced liver fibrosis and response to hepatitis C virus therapy: a meta-analysis. Hepatology 2014; 60: 1541–50.
- Hanley AJG, Williams K, Gonzalez C, Wagenknecht LE, Stern MP, Haffner SM, et al. prediction of type 2 diabetes using simple measure of insulin resistance. Diabetes 2003; 52: 463-9.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911–30.
- Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 2004; 165[5]: 1499-508.
- Kochupillai N. The physiology of vitamin D: Current concepts. Indian J Med Res 2008; 127: 256-62.
- Kumar D, Farrell GC, Fung C. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: Reversal of hepatic steatosis after sustained therapeutic response. Hepatology 2002; 36: 1266–72.
- Le Roith D, Zick Y. Recent advances in our understanding of insulin action and insulin resistance. Dia Care 2001; 24: 588-97.
- Lips P. Vitamin D physiology. Prog Biophys Mol Biol 2006; 92: 4-8.
- Maeno T, Okumura A, Ishikawa T. Mechanisms of increased insulin resistance in non-cirrhotic patients with chronic hepatitis C virus infection. J Gastroenterol Hepatol 2003; 18: 1358- 63.
- Mariana CS, Telma ES, Maria LA, Mara SP, Letica MW, David AG, et al. Factors associated with 25-hydroxyvitamin D levels in patients with liver cirrhosis. Ann Hepatol 2015; 14: 99-107.
- Marra F. Hepatic stellate cells and the regulation of liver inflammation. J Hepatol 1999; 31: 1120–30.

- Miller FD, Abu Raddad LJ. Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. Proc Natl Acad Sci USA 2010; 107[33]: 14757-62.
- Paradis V, Perlemuter G, Bonvoust F. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 2001; 34: 738–44.
- Perrillo R. The role of liver biopsy in hepatitis C. Hepatology 1997; 26[1]: 57-61.
- Petta S, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology 2010; 51: 1158–67.
- Poynard T, Cacoub P, Ratziu V, Myers RP, Dezailles MH, Mercadier A, et al. Fatigue in patients with chronic hepatitis C. J Viral Hepat 2002; 9[4]: 295-303.
- Putz-Bankuti C, Pilz S, Stojakovic T, Scharnagl H, Pieber TR, Trauner M, et al. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. Liver Int 2012; 32: 845–51.
- Sporea I, Sirli R, Popescu A, Danila M. Acoustic Radiation Force Impulse (ARFI) a new modality for the evaluation of liver fibrosis. Med Ultrason 2010; 12: 26-31.
- Stephen L,Timothy R. The Natural History of Hepatitis C Virus (HCV) Infection. Int J Med Sci 2006; 3[2]: 47–52.
- Stokes CS, Volmer DA, Grunhage F. Vitamin D in chronic liver disease. Liver International 2013; 33: 338-52.
- Streiff MB, Mehta S, Thomas DL. Peripheral blood count abnormalities among patients with hepatitis C in the United State. Hepatology 2002; 35: 947-52.
- Su-Mei L, Guang-Xia L, Dong-Mei F, Yu W, Li Qun D. Liver fibrosis evaluation by ARFI and APRI in chronic hepatitis C. World J Gastroenterol 2014; 20[28]: 9528–33.
- Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and diabetes. Endocrinology and Metabolism Clinics of North America 2010; 39: 419–46.