Impact of Hepatic Steatosis on Response to Antiviral Therapy in Egyptian Patients with Chronic Hepatitis C

Atef Abo Alsoud Aly[1], Gamal Saad El-Deeb[1], Basam Mohamed Masoud[1], Emad Mohamed Salem[2]

[1]*Tropical Medicine Department, Faculty of Medicine, Menofia University, Egypt*
[2]*Hepatology Unit, Mansoura General Hospital, Egypt*

Corresponding Author: ***Emad Mohamed Abd El-Kawy Salem***

E mail: *Dr.emad.hep@hotmail.com*

Key words:
Hepatic steatosis, Chronic hepatitis C virus

Background and study aim: Hepatic steatosis in hepatitis C virus (HCV) infected patients has been shown to enhance the progression of liver fibrosis and decrease the response to antiviral therapy. The current study is designed to investigate the impact of hepatic steatosis on the outcome of pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C genotype 4.

Patients and Methods: A total number of 200 patients were selected from 270 patients who were referred to HCV Treatment Unit of New Mansoura General Hospital from February 2012 to August 2013 after taking an informed consent. They were 129 males and 71 females, their ages ranged from 25 to 55 years (mean value, 35.5±15.2). They had proven chronic hepatitis C virus based on history of exposure, clinical manifestations, positive anti-HCV antibody, positive HCV viremia, and liver biopsy findings suggestive of chronic hepatitis C.

Results: *Group I*: included 100 patients (70 men and 30 women; mean age of 42.9±12 years) without liver steatosis. *Group II*: included 100 patients (59 men and 41 women; mean age of 45.23±11 years) with liver steatosis. In terms of steatosis grading using the NAS and METAVIR scoring systems, 50% had no staetosis while 8.5% had mild staetosis, 18.5% had moderate steatosis and 23% had severe steatosis. Body mass index of patients receiving interferon is significant between both groups. Hepatomegaly shows significant values between both groups. Platelets count, ALT, AST, S.Cholesterol & S.Triglycerides levels has statistically significant differences between group I (non steatotic) and group II (steatotic). There is statistically significant difference between both groups on necro-inflamatory activity grades. High statistical significance difference between grading of steatosis and Necro-inflammation. Statistical significance difference between grading of steatosis and fibrosis stages. Statistical significance difference between both groups at SVR and Steatosis has a negative effect on SVR by comparison to non steatotic group. High degree of hepatic steatosis has a negative impact on pagylated interferon and ribavirin therapy in chronic HCV genotype 4 minimizing sustained virologic response rates.

Conclusion: Our study confirms that hepatic steatosis correlates with BMI, S.cholesterol, S.triglycerides, fibrosis, necro-inflammatory stages and has a negative impact on response to antiviral therapy.

INTRODUCTION

The incidence of hepatitis C virus (HCV) infections is falling in some countries, however the burden of the disease in Egypt continues to rise. It has been estimated that, by 2030, HCV will cause substantially higher morbidity and mortality than HIV. Chronic Hepatitis C (CHC) occurs in 70% to 80% of those who contract the virus, 20% of whom will progress to cirrhosis within 2-3 decades; a quarter of these will develop decompensated liver disease, hepato-cellular carcinoma (HCC) and will need liver transplantation. A recent study has shown that HCV infected persons have three times higher death rates than those of age-matched general population [1].

In patients with chronic HCV infection, steatosis is attributable to a variable combination of the mechanisms considered to play a role in the pathogenesis of NAFLD; insulin resistance in the obese and in the lean subject along with a direct effect of HCV on hepatic lipid metabolism that leads to triglyceride accumulation through inhibition of export proteins that are required for very low density lipoprotein (VLDL) assembly and secretion [2].

Hepatic steatosis resulting from host metabolic factors or via direct viral effect has been associated with increased fibrosis, regardless of genotype. Worsening hepatic steatosis also is associated with increased periportal necrosis, hepatocyte apoptosis, and fibrosis progression [3].

Leptin also may mediate fibrogenesis. This satiety hormone has been shown to be profibrogenic and up-regulation of leptin signaling may lead to fibrosis progression. In CHC genotype 1 patients there appears to be a correlation between serum leptin levels, steatosis, and fibrosis [4].

Steatosis seems to reduce the likelihood of obtaining sustained virological response (SVR) from HCV medications at least in people with HCV non-3 type, the impact of steatosis on SVR in genotype 3 is less clear [5].

The risk of non-response to antiviral therapy was increased 2-fold if significant steatosis or steatohepatitis (SH) were present on biopsy. Furthermore, there is a significant difference in overall SVR between groups. It is noteworthy that the effect of steatosis/SH on SVR was greatest in the genotype 2 or 3 patients [6].

It is become clear that there is direct viral mechanisms involved in the development of steatosis in people infected with HCV genotype 3, although in genotype other than 3 other co-factors such as high BMI, heavy alcohol intake, elevated blood lipids, glucose intolerance and diabetes greatly promotes the development of steatosis[5].

Besides obesity, type 2 diabetes mellitus (DM) and hypertriglyceridemia have also been associated with hepatic steatosis in patients with non-alcoholic fatty liver disease [7].

Aim of the work:

The current study is designed to investigate the impact of hepatic steatosis on the outcome of pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C.

PATIENTS AND METHODS

A total number of 200 patients were selected from 270 patients who were referred to HCV Treatment Unit of New Mansoura General Hospital from February 2012 to August 2013 after taking an informed consent. They were 129 males and 71 females, their ages were ranging from 25 to 55 years with mean value of 35.5±15.2 with proven chronic hepatitis C virus based on history of exposure, clinical manifestations, positive anti-HCV antibody, positive HCV vireamia, and liver biopsy findings suggestive of chronic hepatitis with documented chronic hepatitis C

According to the histopathological grade of steatosis, enrolled patients were classified into two groups:

Group I: Included 100 patients (70 men and 30 women; mean age of 42.9±12 years) without liver steatosis.

Group II: Included 100 patients (59 men and 41 women; mean age of 45.23±11 years) with liver steatosis.

Exclusion criteria:

Patients were excluded from the study if one or more of the following conditions were present:

1- Previous IFN therapy.
2- Evidence of other liver diseases including; hepatitis B, autoimmune hepatitis, alcoholic liver disease or drug induced hepatitis.
3- Decompensated liver disease with a history of variceal hemorrhage, ascites, or hepatic encephalopathy.
4- Concurrent cardiac or respiratory diseases or diabetes mellitus.
5- Patients with a leukocyte count lower than 4000/mm^3, neutropenia (<1500 cells/mm^3), a hemoglobin level lower than 12 g/dL for women and lower than 13 g/dL for men, thrombocytopenia (<90,000 cells/mm^3).
6- Creatinine concentration 1.5 times the upper limit of normal.
7- Neoplastic disease, unstable thyroid dysfunction, unstable psychiatric disorder or history of any organ transplantation.
8- Current therapy with immune modulatory agents or immunosuppressive within the last 6 months.

All the included patients were subjected to the following:

Full medical history, thorough clinical examination, laboratory investigation (CBC, ALT, AST, S. bilirubin, serum albumin, prothrombin time, INR,

HCV antibody, HBsAg, quantitative PCR before treatment and at weeks 12, 24, 48 and 24 weeks after end of treatment, s. cholesterol, s. triglycerides, s.TSH, fasting blood sugar, renal functions, s. Alkaline phosphatase, ANA, AFP and IHA for bilharziasis), imaging (abdominal ultrasonography) and liver biopsy.

Statistical analysis:

Data were collected, tabulated and statistically analyzed by computer using SPSS version 16. The following tests were used; arithmetic mean, standard deviation (SD), standard student "t test", Chi square Test (X^2), sensitivity, specificity, accuracy, positive predictive value, negative predictive value, linear correlation coefficient [r], Roc curve (Receiver operating characteristic curve) and significance of results (P value) .

RESULTS

Chronic HCV genotype 4 is associated with hepatic steatosis which is mostly metabolic associated with elevated BMI, triglycerides and cholesterol levels.

- Body mass index of patients has statistical significant between both groups as shown in Table (1).
- Hepatomegaly shows significant values between both groups.
- Platelets count, ALT, AST, s.cholesterol and s. triglycerides levels has statistically significant difference between group I (non steatotic) and group II (steatotic) group as shown in Table (2).
- Statistical significance between both groups on necroinflamatory activity grades as shown in Table (3).
- High statistical significance between grading of steatosis and necro-inflammation as shown in Table (4) and Figure (2).
- Statistical significance between grading of steatosis and fibrosis stages as shown in Table (4) and Figure (3).
- Statistical significance between both groups at SVR and steatosis has a negative effect on SVR by comparison to non steatotic group as shown in Table (5).
- High degree of hepatic steatosis has a negative impact on pagylated interferon and ribavirin therapy in chronic HCV genotype 4 minimizing sustained virologic response rates.

Table (1): Gender, age and BMI distribution among studied groups.

	Group I		Group II		X^2	P.Value
	N	%	N	%		
Male	70	70	59	59	2.642	> 0.05
Female	30	30	41	41		
Total	100		100			
	Mean	SD	Mean	SD		
BMI	27.6	2.19	29.54	1.2	t- test= 7.77	< 0.05
Age	42.9	12	45.23	11	t- test= 1.43	> 0.05

Table (2): Other laboratory findings before starting treatment in two groups.

	Group I	Group II	t- test	P.Value
AST(up to 40 U/ml) -Mean -Range	 55±13 25-70	 63±19 30-87	3.48	< 0.05*
ALT(up to 45 U/ml) -Mean -Range	 57±17 32-82	 66±21 40-103	3.33	< 0.05*
Serum Albumin (3.5-5gm/dl) -Mean -Range	 4.2±0.9 3.2-5	 4±1.1 3-5.2	1.41	> 0.05
Serum Bilirubin (0.5-1.2 mg/dl) -Mean -Range	 0.9±0.2 0.4-1.3	 1±0.49 0.5-1.6	1.89#	> 0.05
Fasting blood sugar(80-110mg/dl) -Mean -Range	 103±0.32 70-115	 110±0.36 80-142	1.45	> 0.05
Serum Creatinine (0.7-1.2 mg/dl) -Mean -Range	 0.9±0.3 0.5-1.4	 1±0.42 0.7-1.5	1.94	> 0.05
AFP(up to 10 ng/ml) -Mean -Range	 8±4.2 4-20	 9±3.2 5-31	1.89	> 0.05
TSH (0.3-5 ul/ml) -Mean -Range	 2.5±1.48 0.2-6.7	 2.9±1.39 0.3-8.1	1.97	> 0.05
HCV RNA(undetected level up to 15 -Mean -Range	 8,000000±141000 12500 – 15,000000	 7,650000±133000 11780 – 20,050000	18.06#	> 0.05
HBS Ag (negative)	negative	negative		
ANA (negative)	negative	negative		
Alkaline phosphatase (21-92 mg/dl) -Mean -Range	 42±12 18 - 85	 45±16 20 – 90	1.5	> 0.05
Cholesterol (up to 200 mg/dl) -Mean -Range	 160±43 90 - 195	 180±52 110 – 290	2.96#	< 0.05*
Triglycerides(up to 150 mg/dl) -Mean -Range	 110±52 80 - 155	 130±62 90 - 210	2.47#	< 0.05*

Mann Whitney test.

Table (3): Necro-inflammatory activity stages and fibrosis grades in both groups.

	Group I		Group II		X^2	*P.Value*
	No	%	No	%		
Activity						
-A0	12	12	5	5		
-A1	29	29	25	25	7.945	< 0.05*
-A2	38	38	33	33		
-A3	21	21	37	37		
Fibrosis						
-F0	5	5	4	4		
-F1	18	18	14	14		
-F2	29	29	23	23	4.980	> 0.05
-F3	27	27	24	24		
-F4	21	21	35	35		
Steatosis						
-Mild			17	17		
-Moderate			37	37		
-Severe			46	46		

Table (4): Grading of steatosis and necro-inflammatory with fibrosis stages in group II (steatosis group).

	Mild Steatosis		Moderate Steatosis		Sever Steatosis		X^2	*P.Value*
	No	%	No	%	No	%		
Activity								
-A0	4	23.5	1	2.7	0	0		
-A1	6	35.3	16	43.2	3	6.5	33.694	<0.001*
-A2	4	23.5	10	27	19	41.3		
-A3	3	17.6	10	27	24	52.2		
Fibrosis								
-F0	2	11.8	2	5.4	0	0		
-F1	6	35.3	5	13.5	3	6.5		
-F2	4	23.5	10	27	9	19.6	17.883	< 0.05*
-F3	3	17.6	9	24.3	12	26.1		
-F4	2	11.8	11	29.7	22	47.8		

Table (5): Virological response (VR) at 12, 24, 48 weeks and SVR in both groups.

	Group I		Group II		X^2	P.Value
	N	%	N	%		
PCR at 12 weeks						
-Responder	85	85	75	75	3.125	> 0.05
-Non responder	15	15	25	25		
PCR at 24 weeks						
-Responder	80	94.1	67	89.3	1.222	> 0.05
-Non responder	5	5.9	8	10.7		
PCR at 48 weeks						
-Responder	77	96.3	64	95.5	0.049	> 0.05
-Non responder	3	3.8	3	4.5		
SVR						
-Responder	71	92.2	51	79.7	4.699	< 0.05*
-Non responder	6	7.8	13	20.3		

* Significant P < 0.05

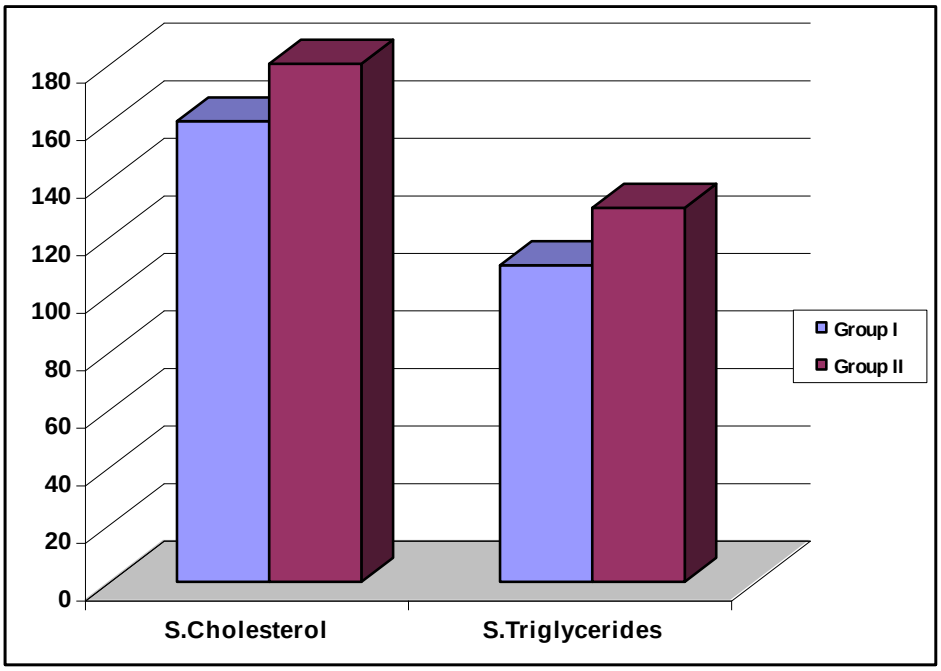

Figure (1): Cholesterol and triglycerides values in group I and group II.

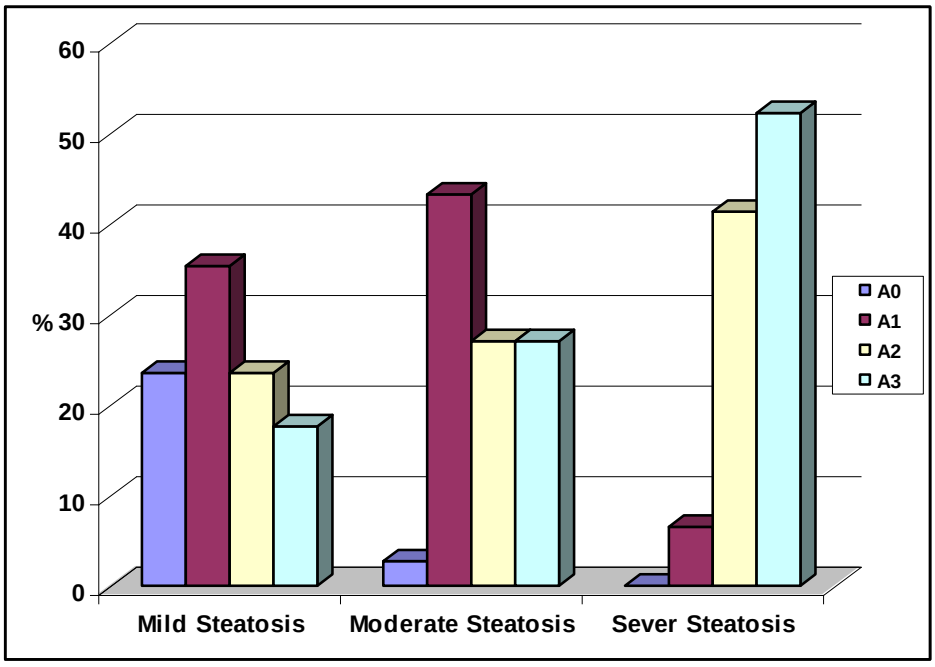

Figure (2): Necro–inflammatory stages in relation to degrees of steatosis.

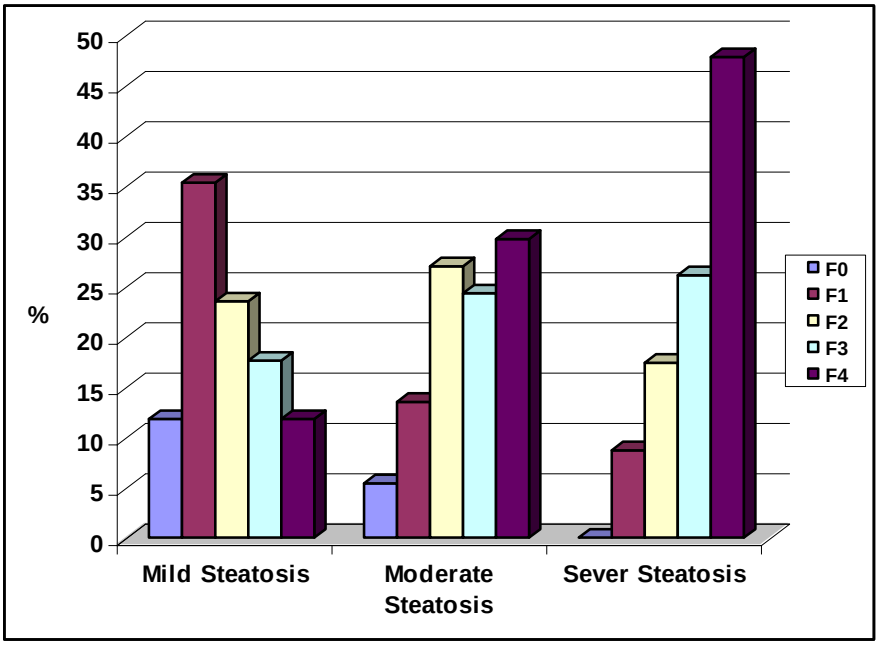

Figure (3): Fibrosis stages in relation to degrees of steatosis.

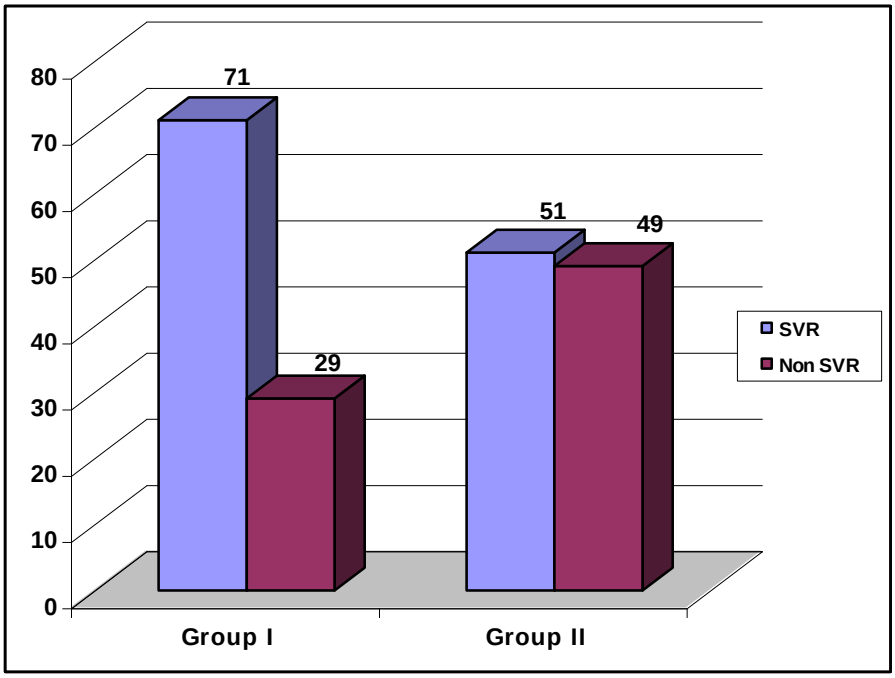

Figure (4): SVR and non SVR values in group I (non steatotic) and group II (steatotic).

DISCUSSION

In the current work, there were a significant corelation between BMI and steatosis so it means that steatosis in HCV genotype 4 is metabolic as shown in table (1) and this is in agreement with El-Zayadi et al. [8] who concluded that steatosis was independently associated with diabetes

mellitus as well as increased BMI. These findings support the proposed causal relation to metabolic factors rather than to cytopathic effect of HCV genotype 4.

Our results revealed statistical significance relation between levels of cholesterol and triglycerides and presence of steatosis as shown in Table (2) and Figure (1). Also our findings are in accordance with Sanyal et al. [9], who reported that the presence of steatosis in HCV genotype 4 infected patients was strongly associated with obesity, diabetes mellitus and abnormal lipid profile.

Our results revealed statistical significant relation concerning AST & ALT levels with patients of group I compared with those of group II ($P<0.05$) as shown in Table (2) and this is in agreement with Minerva [10] mentioned that liver enzymes levels are raised in case of steatosis.

In our series, there was no significant correlation between steatosis and viral load as shown in table (2) and this is an agreement with El-Zayadi et al. [8] who concluded that the presence of steatosis in 55.6%, 52.6%, 56.3% and 60% of genotype 4 patients with very low, low, moderate and severe viral load respectively. This may reflect the absence of association between viral load and steatosis in genotype 4 patients as the proportion of patients with steatosis did not show significant increase with the increase in viral load.

In the present study, there was a significant correlation between stage of fibrosis and steatosis level as shown in Table (4) and Figure (3) and this in accordance with Lonardo et al. [11] who revealed that steatosis is a definite cofactor of chronic hepatitis C which accelerates the progression to end-stage liver disease.

In our study there is a high significant correlation between steatosis and necro-inflamtion as shown in Table (4) and Figure (2) and this in accordance with Lonardo et al. [12] who declared that Presence of steatosis was also correlated with necro-inflammatory activity in nonalcoholic steatohepatitis as well as chronic hepatitis C.

Our study shows that steatosis has a negative effect on SVR by comparison to non steatotic group as shown in Table (5) and Figure (4) and this is in accordance with Fried et al. [13] who concluded that advanced hepatic fibrosis is a negative predictor of SVR to therapy Everson et al. [13].

Our study shows that there is a good correlation between direct fibrosis progression rate and histological activity because fibrosis might be a result of the necro-inflammatory activity as shown in Table (4,5) and this accordance with Mendes et al. [14] who found good correlation between direct fibrosis progression rate and histological activity because fibrosis might be a result of the necro-inflammatory activity.

CONCLUSION

There is strong association between the degree of liver steatosis and fibrosis staging. Although patients with high grading scores tended to have more steatosis; there is a statistical significant correlation between the degree of steatosis and the necro-inflammatory grade

Hepatic steatosis and obesity were predictors of poor response to pegylated IFN and ribavirin therapy in CHC genotype 4.So reduction of the weight before treatment is an important to improve sustained response rates. The evaluation of hepatic steatosis is not only useful for prediction of treatment outcome, but also important for investigation of new approaches toward overcoming the IFN resistance in patients with chronic hepatitis C.

Ethical approval: Approved.

Funding: None.

Conflict of interest: None.

REFERENCES

1. Brok J, Gluud LL, Gluud C. Meta-analysis: Ribavirin plus interferon vs. interferonmonotherapy for chronic hepatitis C–an updated Cochrane review. *Aliment Pharmacol Ther* 2010; 32:840-50.
2. Fried MW, Hadziyannis SJ, Shiffman ML, Messinger D, Zeuzem S. Rapid virological response is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection. *Journal of Hepatology* 2011; 55: 69-75.
3. Izumi N, Asahina Y, Kurosaki M. Predictors of Virological Response to a Combination Therapy with Pegylated Interferon Plus Ribavirin Including Virus and Host Factors Hindawi Publishing Corporation. *Hepatitis Research and Treatment* 2010; 2010: 703602.
4. Ferenci P, Laferl H, Scherzer Tm, Gschwantler M, Maieron A, Brunner H, et al. Peginterferon Alfa-2a and Ribavirin for 24 Weeks in Hepatitis C Type 1 and 4 Patients With Rapid Virological Response. *Gastroenterology* 2008; 135: 451-458.

5. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The Diagnosis and Management of Non-Alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; 55(6): 2005-2023.
6. Rodriguez–Torres M, Stoehr A, Gane EJ، Serfaty L, Lawitz E, Zhou A, et al. Combination of Vaniprevir With Peginterferon and Ribavirin Significantly Increases the Rate of SVR in Treatment-Experienced Patients With Chronic HCV Genotype 1 Infection and Cirrhosis Clinical. *Gastroenterology and Hepatology* 2014; 12:1029–1037.
7. Zhu Q, Han Q, Zhang P, Yang C, Zeng X, Chen Y, et al. Statin therapy improves response to interferon alfa and ribavirin in chronic hepatitis C: A systematic review and meta-analysis. *Antiviral Research* 2013; 98: 373–379.
8. El-Zayadi A, Attia M, Barakat EM, Zalata K, Saeid A, Hamdy H et al. Non-alcoholic fatty liver disease in patients with HCV genotype 4. *Gut* 2007; 56: 1170-1171.
9. Sanyal AJ, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Stravitz RT, et al. Nonalcoholic fatty liver disease in patients with hepatitis C is associated with features of the metabolic syndrome. *Am J Gastroenterol* 2003; 98(9): 2064-71.
10. Minerva L. Steatosis, insulin resistance and fibrosis progreesionin chronic hepatitis C. *Gastroenterol Dietol* 2006; 52 (2): 125-34.
11. Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: Mechanisms and significance forhepatic and extrahepatic disease. *Gastroenterology* 2004; 126(2): 586-97.
12. Lonardo A, Loria P, Adinolfi LE, Carulli N, Ruggiero G. Hepatitis C and steatosis: a reappraisal. *J Viral Hepat* 2006; 13: 73-80.
13. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975-82.
14. Mendes LS, Nita ME, Ono-Nita SK, Mello ES, da Silva LC, Alves VA, et al. Prognostic factors for progression of liver structural lesions in chronic hepatitis C patients. *World J Gastroenterol* 2008; 14 (16): 2522-2528.

Peer reviewer: Tarik Zaher, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.
Editor: Mohamad Emara: Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt