IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 5 Ver. IV (Sep - Oct. 2015), PP 46-52
www.iosrjournals.org

Biochemical Evaluation of the Metabolic Abnormalities Associating with Hepatitis C virus Patients

E. El Agroudy1 , Omnia .E. STEAT2 , Mostafa .A. Hussien3 , Nahla.H.Anber4 , Shereen .M. Attallah5

1 (Department of Medical Biochemistry, Faculty of Medicine, Mansoura University, Mansoura, Egypt)
2(Department of Internal Medicine, Faculty of Medicine/ Mansoura University, Mansoura, Egypt)
3 (Department of Chemistry, Faculty of Science/ Port Said University, Port Said, Egypt)
4 (Fellow of Biochemistry, Emergency Hospital, Mansoura University, Egypt)
5 (Department of Chemistry, Faculty of Science/ Port Said University, Port Said, Egypt)

Abstract: *Hepatitis C virus (HCV) is one of the main causes of liver disease worldwide, with more than 150 million of persons chronically infected, at risk of developing liver cirrhosis and cancer. Moreover, HCV infection is associated with glucose and lipid metabolism disturbances. HCV can induce insulin resistance through several mechanisms. Indeed, the virus interferes with insulin signaling both directly and indirectly, inducing the production of pro-inflammatory cytokines. In this study, we assess the metabolic abnormalities in patients with chronic hepatitis C virus infection, in order to estimate the potential link between HCV patients and metabolic syndrome. The study included 65 subjects, 55 HCV patients&10 healthy individuals with matched age &sex. Determination of oral glucose tolerance test (OGTT), liver enzymes, lipid profile and serum insulin were done for all subjects. Total cholesterol, triglycerides and LDL levels were lower in HCV with DM when compared to control group (statistically significant difference p<0.001). HOMA -R significant increase in diabetic group (**p=0.02**) while HOMA –B showed a significant decrease in diabetic group and impaired OGTT (p=0.01 and 0.01 respectively). The results suggest a strong interrelationship between HCV infection and metabolic disorders, which is likely implicated in the progression both of liver damage and of the atherogenic process.*

Keyword: *Hepatitis C, metabolic syndrome, Insulin resistance, lipid metabolism*

I. Introduction

The liver plays a pivotal role in nutrient and hormone metabolism; therefore, several metabolic abnormalities are common in liver disease. Chronic infection with hepatitis C virus (HCV) is a major cause of progressive liver damage, whose long term sequels include cirrhosis and primary hepatocellular carcinoma (**Nicderau et al., 1998**). However, the rate of histological and clinical progression of chronic hepatitis is variable, depending on the presence of several factors (**Rubbia-Brant, 2000**). Chronic hepatitis C (CHC) has many features which suggest that this disease must be viewed not only as a viral disease, but also as a metabolic liver disease which implies: insulin resistance (IR) (**Fartoux et al, 2005**), high prevalence of steatosis (**Adinolfi et al, 2005**), increased prevalence of impaired glucose tolerance (**Lecube et al, 2007**), type 2 diabetes mellitus (**Mehta et al, 2000**) and changes in lipid metabolism (**Perlemuter et al, 2002**). These findings together suggest that chronic HCV infection is closely related to the metabolic syndrome (MS). Accordingly, CHC should be divided into CHC with MS and CHC without MS.

The prevalence of DM in patients with chronic hepatitis and liver cirrhosis due to HCV infection is higher than that in patients with chronic liver disease due to other causes including B virus (HBV) infection (**Fraser et al., 1996; Caronia et al., 1999; Mason et al., 1999 and Knobler et al., 2000**). The incidence of DM in HCV infected recipients after liver transplantation is higher than that in patients with other liver disease (**Knober et al., 1998**). These studies suggested the possibility that DM is another extra hepatic disorder associated with HCV infection (**Konrad et al., 2000; Mason et al., 2003**).Despite strong association between HCV-infection and glucose metabolism, the link with other features of MS is not clear and sometimes, counterintuitive. While MS is associated with high triglycerides and low HDL-cholesterol, in chronic hepatitis C, decreased levels of cholesterol and LDL are observed. Moreover, achieving viral clearance is associated with an increase in LDL and cholesterol, which requires use of lipid lowering therapies in up to 1/3 of individuals (**Corey KE, et al., 2009**). Hepatitis C infection was associated with lower triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL) and higher high density lipoproteins (HDL) levels. Several lines of evidence have indicated that HCV or its viral components are able to induce derangements of host lipid metabolism (**Marchesini et al., 2003 and Shaheen et al., 2007**). Interactions between chronic hepatitis C virus (HCV) infection and lipid metabolism have been suggested (**Serfaty et al., 2001**). Some studies have reported a higher prevalence of hypocholesterolemia and hypobetalipoproteinemia in HCV infected patients compared

with control groups (Maggi et al., 1996). Although changed serum lipid and Apo lipoprotein composition is commonly found in patients with chronic liver disease of any etiology, the relationship between HCV and lipid metabolism seems to be more specific: binding of HCV particles to human high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) has been described (**Thomssen et al., 1993**). Moreover the LDL receptors could permit the entry of HCV in hepatocytes (**Monazahian et al., 1999 and Agnello et al., 1999**).

In this study, we assess the metabolic abnormalities in patients with chronic hepatitis C virus infection, in order to estimate the potential link between HCV patients and metabolic syndrome.

II. Subjects and methods

II.1 subjects

This study included 65 subjects, 55 HCV patients, their age range from 29 to 55 .they were 31(56.4%) male and 24 (**43.6%**) female and 10 healthy individuals 6 male and 4 female, their ages ranged from 29 to 55, all patients were selected from Internal Medicine Centre, Mansoura University, the control subject were collected from donor blood bank Mansoura university. Serum glucose levels, Liver enzymes tests (AST and ALT),Complete lipogram and Serum insulin were tested .The control subjects had normal liver functions ,normal oral glucose tolerance level and normal lipid levels.

II.2Blood sampling:

Five ml venous blood sample were withdrawn from every subject by aseptic venipuncture from an anticubital vein in a fasting state. The blood was left to clot in plain polypropylene tube at 25^{o}C for 30 min. then centrifuged and the separated serum was divided into 2 tubes; the first tube was used for the assay of serum fasting glucose level (**Trinder, 1969**), liver function tests including serum ALT and AST, serum cholesterol, triglycerides, LDL and HDL **(Artiss et al., 1989),** using automatic auto analyzer Hitachi 902 Boehringer Manneim, and the second tube was stored at -70^{o}C for the assay of serum insulin. The subjects then were given an oral glucose load (75 g glucose in 200ml water).

Then, 4 samples were withdrawn from each subject every 30 min., and the separated serum was used for the assay of serum glucose level using automatic auto analyzer **(Hitachi 902 Boehringer Manneim**) to establish OGTT.

II.3 Statistical Analysis:

Data were obtained using Statistical package for social Sciences (SPSS) version 19.0 software. Data were expressed as means ± standard deviation (SD) .Results of HCV patients and control subjects were performed using chi- square analysis, way a nova, and independent t-test. Correlation between parameters was determined by Pearson's correlation coefficient (**r**). Chi square and odds ratio were calculated with 95% confidence interval .A p- value less than 0.05was considered statistically significant.

III. Results

Table (1): Biochemical Features of studied subjects with control

	Control	HCV pt. with Normal OGTT	HCV pt. with Impaired OGTT	HCV Pt. With D.M	P Value
	N=10	N=10	N=20	N=25	
	Mean ±SD				
ALT(mg/dl)	36.2 ±7.3	34.8±8.8	36.5±7.4	58.9±16.9	<0.001*
AST(mg/dl)	33.2±7.8	35.7±6.6	34.3±7.2	51.9±17.8	<0.001*
T. cholesterol (mg/dl)	165.10± 16	173.7±15.5	140.05±12.3	107.7±7.7	<0.001*
Triglycerides (mg/dl)	93.3±12.9	109.6±13.5	62.5±8.1	42.08±7.3	<0.001*
HDL(mg/dl)	51.5±7.4	48±3.2	57.1±7.8	75.5±8.7	<0.001*
LDL(mg/dl)	96.0± 12.0	101.0±17.7	71.00±11.5	24.08±7.4	<0.001*

P * value: one way a nova (**F**)

P <0.05 is considered as statistically significant

In table (1) biochemical parameters such as alanine aminotransferase (ALT) enzyme, aspartate aminotransferase (AST) enzyme , HDL high density lipoprotein ,LDL low-density lipoprotein, triglycerides, total cholesterol were tested for hepatitis C virus (HCV) patients (n=55),as well as control subjects (n=10).

.

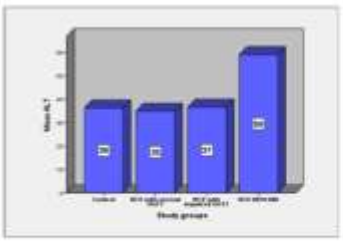

Figure (1): Mean levels of ALT among studied groups

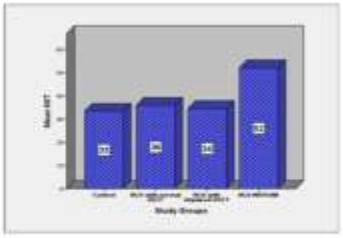

Figure (2): Mean levels of AST among studied groups

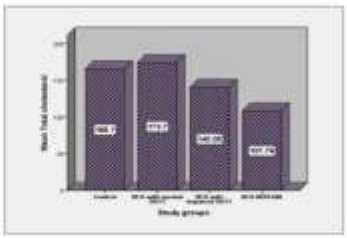

Figure (3): Mean levels of total cholesterol among studied groups

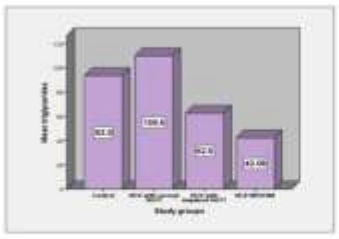

Figure (4): Mean levels of triglycerides among studied groups

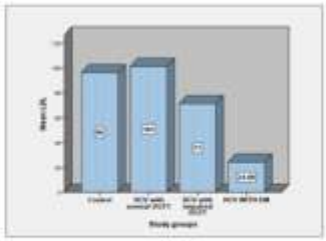

Figure (5): Mean levels of HDL among studied groups

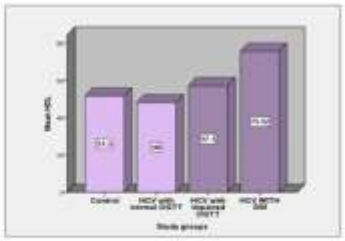

Figure (6): Mean levels of LDL among studied groups

Table (2): Oral glucose tolerance test in various studied group

I	Data	0 min	30 min	*60* min	90 min	120 min
	Healthy Control Group	81.67±10.27	97.33±10.63	123.33±2.36	102.33±2.06	88.2±2.30
(A)	HCV Pt. With Normal OGTT	84.07±9.13	132.39±25.96	137.64±26.32	113.53±36.10	98.7±23.90
(B)	HCV Pt. With Impaired OGTT	113.83±2.48	155.33±7.87	183.67±20.02	176.17±24.84	151.8±12.20
(C)	HCV Pt. With diabetes	106.60±40.01	185.80±52.76	233.40±55.36	228.60±90.14	197±106.80
P1		0.55	0.00	0.16	0.42	0.24
P2		0.00	0.00	0.00	0.00	0.00
P3		0.00	0.00	0.00	0.00	0.00
P4		0.00	0.03	0.00	0.00	0.00
P5		0.01	0.00	0.00	0.00	0.00
P6		0.01	0.00	0.00	0.00	0.00

P significant at $p<0.05$

P_1: group (**A**) versus control, P_2: group (**B**) versus control, P_3: group (**C**) versus control, P_4: group **(B)** versus group (**A**), P_5: group (**C**) versus group (**A**) and P_6: group (**C**) versus group (**B**)

As regards peak serum glucose levels were obtained at 60 min in all three groups (normal OGTT, impaired OGTT and DM). There was significant difference among three groups in the pattern of serum glucose levels at corresponding time points ($p<0.05$).

Table (3): Insulin levels in the studied groups in compared to control group.

	Data		Fasting Insulin (µu/ml)
	Healthy Control Group	M ± SD	11.78 ± 7.10
(A)	HCV pt. With Normal OGTT		7.24 ± 4.35
(B)	HCV Pt. With OGTT		11.51 ± 7.89
(C)	HCV Pt. With Diabetes		11.83 ± 3.11
P1			0.04
P_2			0.95
P_3			0.99
P_4			0.05
P5			0.02
P6			0.93

P significant at <0.05

P_1: group (**A**) versus control, P_2: group (**B**) versus control, P_3: group (**C**) versus control, P_4: group (**B**) versus group (**A**), P_5: group (**C**) versus group (**A**) and P_6: group (**C**) versus group (**B**).

As regards fasting serum, there was a significant decrease levels in HCV patients with normal OGTT when compared to control group (p=0.4). Also there was a significant increase in HCV patients with impaired OGTT and DM group when compared to HCV patient group with normal OGTT (p=0.05 and p=0.02, respectively). While the other studied groups showed non -significant changes when compared to each other or to the control group.

Table (4): HOMA-R in various studied groups

	Control Group	HCV Pt. With Normal OGTT	HCV Pt. With Impaired OGTT	HCV Patients With D.M
	N=10	N=10	N=20	N=25
	Mean ±SD			
HOMA –R (mmol /l)	2.42± 1.43	2.56±0.75	3.26±2.28	4.86± 1.87
P_1	-	0.72	-	-
P_2	-	-	0.43	-
P_3	-	-	-	0.02
P_4	-	-	0.17	-
P_5	-	-	-	0.00
P_6	-	-	-	0.19

P_1: group (**A**) versus control, P_2: group (**B**) versus control, P_3: group (**C**) versus control, P_4: group (**B**) versus group (**A**), P_5: group (**C**) versus group (**A**) and P_6: group (**C**) versus group (**B**).

From this table, HOMA-R showed non-significant changed patients with normal OGTT or patients with impaired OGTT group when compared to the control group or to each other. HOMA-R showed Significant increase in diabetic group when compared to the control group or to the HCV patients with normal OGTT (P= 0.02 and 0.00, respectively).

Table (5): HOMA-B in various studied groups

	Control Group	HCV With Normal OGTT	HCV. With Impaired OGTT	HCV With D.M
	N=10	N=10	N=20	N=25
	Mean ±SD			
HOMA-B (mIU/mmol)	256.74± 141.25	151.10 ±134.13	81.13 ± 53.46	50.48 ± 33.69
P_1	-	0.07	-	-
P_2	-	-	0.1	-
P_3	-	-	-	0.01
P_4	-		0.19	-
P_5	-			0.08
P_6	-	-		0.25

P_1: group (A) versus control, P_2: group (B) versus control, P_3: group (C) versus control, P_4: group (B) versus group (A), P_5: group (C) versus group (A) and P_6: group (**C**) versus group (**B**)

From this table, the HOMA-B showed a significant decrease in HCV patients with impaired glucose tolerance and diabetic group when compared to the healthy control group (P=0.01 and 0.01, respectively'). The other groups showed no significant change when compared to the control group or to each other.

IV. Discussion

Hepatitis C virus (HCV) is one of the main causes of liver disease worldwide ,with more than 150 million of persons chronically infected, at risk of developing liver cirrhosis and cancer. Moreover, HCV infection is associated with glucose and lipid metabolism disturbances. Alterations of glucose metabolism, *i.e.*, impaired fasting glucose, impaired glucose tolerance and diabetes mellitus (DM), have reached pandemic proportions in western countries. Their keystone is insulin resistance (IR), they are closely linked to obesity and increase the risk of cardiovascular events. Given the prevalence of HCV infection and of these glucose metabolism disturbances, their frequent relationship is not unexpected: however, physiopathologically, it is not only coincidental. In fact, the virus causes IR and predisposes to DM (**Fartoux et al, 2005**).

Although hepatitis C virus targets the liver, it has become increasingly evident that HCV can induce diseases of many organs. Recently**,** much attention is drawn to metabolic disorders in HCV infection. First**,** hepatic steatosis and derangement in lipid metabolism have been found characteristic of HCV infections, and later on, a correlation was noted between HCV infection and diabetes as well as insulin resistance ***(Kazuhiko, 2006) .***

In this study, we observed elevated levels of both ALT and AST in the three studied groups with much more significant elevation in the diabetic group. Similarly, ***Maeno et al. (2003)*** reported the same results. Also, ***Yazicioglu et al, (2004)*** stated that there was no correlation between serum ALT and AST with the degree of glucose intolerance. ***Konrad et al. (2000)***, investigated the link between HCV infection and glucose intolerance and found that serum AST and ALT were negatively correlated to insulin sensitivity. Also he stated that AST, more than ALT, influenced insulin resistance. It's well known that the ratio of AST to ALT changes in the course of chronic hepatitis and AST dominates in patients in advanced stages. It is also known that AST is contained not only in hepatocytes but also in muscle tissue which plays a major role in glucose uptake. Although the possible factors have not been identified, it is speculated that AST represents hot only the magnitude of liver cell injury but also unknown factors which could be related to insulin resistance *(***Maeno et al., 2003***)*.

Serum cholesterol levels showed a significant decrease in HCV group with DM & insignificant change in other diseased groups when compared to control group. Serum triglycerides levels were significantly lower in HCV group with diabetes and insignificantly changed in normal OGTT and impaired OGTT group when compared to the control group. HDL was higher in HCV group with DM compared to control group with statistically significant difference (P<0.001). LDL was lower in HCV group with DM and impaired OGTT compared to control group with statistically significant difference (P<0.001). Similarly, **Moriya *et* al.** ***(2003)*** stated that patients infected with HCV have abnormalities in serum lipids, such as hypocholesterolemia and abnormal level of Apo lipoprotein in serum.

There are several identified mechanisms whereby HCV may alter lipid metabolism. Firstly, HCV core protein has been shown to directly inhibit the function of microsomal triglyceride transfer protein, a major regulator of hepatic assembly, and secretion of nascent triglyceride rich very low density lipoproteins (VLDL).

The latter effect impairs the ability of hepatocytes to assemble and secret VLDL **(*Serfaty et al., 2001*).** Secondly, HCV core protein has been observed to induce mitochondrial injury resulting in oxidative stress. Oxidative stress perturbs lipid peroxidation, thereby contributing to the development of steatosis (**Okuda M et al., 2002**).

Oral glucose tolerance test showed peak plasma glucose levels at 60 min. in all three groups (normal glucose tolerance, impaired glucose tolerance and DM). There was a significant difference among three groups in the pattern of plasma glucose levels at corresponding time points (**P< 0.05**). Fasting serum insulin showed significant decrease in HCV patients with normal OGTT when compared to control group (**P=0.04**). Also, there was a significant increase in HCV group with impaired OGTT and diabetic group when compared with HCV group with normal OGTT (P=0.05 and 0.02, respectively). Their results were in accordance with the results of ***Narita et al. (2004).***They explained the hyperinsulineaemia in the peripheral blood in chronic active hepatitis to be due to diminished hepatic I insulin extraction by the diseased liver, and not to pancreatic hyper secretion.

Petit et al., 2001 reported the lack of correlation between HCV viral load and B-cell function in their study. On the other hand**, Massini et al., 2005**demonstrated a direct cytopathic effect of HCV at islet cell level. HCV infection was present in human pancreatic B-cell which was associated with morphological cell changes and reduced glucose –stimulated insulin release in vitro.

Hui et al., 2003 reported that an increase of fasting insulin and a decrease in insulin sensitivity have been observed in HCV-infected subjects with a moderate or severe degree of hepatic fibrosis .However, HCV infected patients without fibrosis (fibrosis stage 0) also present higher insulin resistance than patients with primary biliary cirrhosis with different degree of hepatic fibrosis (fibrosis stage 1-3) and healthy individuals. These data suggest that HCV is capable of producing an increase in insulin resistance, even before a minimal degree of hepatic fibrosis is present.

The insulin resistance was measured by the homeostatic model assessment of insulin resistance (**HOMA-R**). HOMA-R in CHC patients with DM was significantly higher than in those with both normal and impaired glucose tolerance. Insulin resistance increases with the development of glucose intolerance. Similarly, ***Narita et al. (2004); Maeno et al. (2003); Yaziciotu et al. (2004) and Konrad et al (2000)*** suggested that insulin resistance in patients with CHC due to HCV infection is caused by the liver disease itself. ***Knobler et al. (2000)*** reported that the prevalence of DM in patients with chronic hepatitis and liver cirrhosis due to HCV infection is higher than that in patients with chronic liver disease due to other causes including hepatitis B virus (HBV) infection. Moreover, ***Petit et al. (2001)*** demonstrated that insulin resistance in non-diabetic HCV-infected patients correlates with the staging of liver fibrosis but may occur at an early stage in the course of HCV infection, even in nondiabetic patients.

HCV by itself can induce insulin resistance through disturbing the insulin signaling pathway by HCV proteins. The fact that HCV infection induces insulin resistance by the virus itself may influence the progression of choronic liver disease and open up novel therapeutic approaches ***(*kazuhiko, 2006***)***.*

The B-cell function was assessed by the homeostatic model for assessment of B-cell function (HOMA-B). HOMA- B was significantly lower in diabetic group when compared to other diseased groups. Also, there

was significant decrease in HOMA-B in HCV groups with impaired and normal OGTT when compared to the control group. These results were similar to those reported by ***Narita (2004)*** who stated that HOMA-B decreased in CHC patients with glucose intolerance.

V. Conclusion

Chronic HCV infection induces insulin resistance through disturbing the insulin signaling pathway may influence the progression of chronic liver disease. Chronic HCV patients, hypobetalipoproteinemia occurs even in the early stages of HCV infection ,before the development of liver cirrhosis. The correlation between Apo B levels and HCV viral load seems to confirm the interaction between hepatitis C infection and ß-lipoprotein metabolism.

Reference

[1]. Narita R, Abe S and Kihara Y (2004): Insulin resistance and insulin secretion in chronic hepatitis C virus infection. J Hepatol, 41: 132-138.

[2]. Petit JM, Bour JB and Galland-Jos C (2001): Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol; 35: 279-283.

[3]. Okuda M, Gosert R, Xiao SY, Weinman SA and Lemon SM (2002):Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology, 122, 352

[4]. Adinolfi LE, Durante-Mangoni E and Zampino R (2005):Review article: hepatitis C virus-associates steatosis – pathogenic mechanisms and clinical implications. Aliment PharmacolTher, 22(suppl2): 52-55.

[5]. Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. ProcNatlAcadSci U S A. 1999; 96(22):12766–12771.

[6]. Artiss JD, Stanberg DR and Zak B (1989): Elimination of free glycerol interference in a colorimetric enzyme triglyceride assay. ClinChemActa.; 182: 109-116.

[7]. Corey KE, Kane E, Munroe C, Barlow LL, Zheng H, Chung RT. Hepatitis C virus infection and its clearance alter circulating lipids: implications for long-term follow-up. Hepatology. 2009; 50(4):1030–1037.

[8]. Fartoux L, Paujol-Robert A and Guechat JO (2005): Insulin resistances is a coaure of steatosis and fibrosis progression in chronic hepatitis C. Gut; 54: 1003-8.

[9]. Fraser GM, Harman I, Meller N, Niv Y and Porath A (1996): Diabetes mellitus is associated with chronic hepatitis C but not chronic hepatitis B infection. Israel Journal of Medical Sciences, 32, 526– 530.

[10]. Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW and George J (2003): Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. Gastroenterology; 125: 1695–1704.

[11]. Kazuhiko K (2006)*:* Hepatitis C virus infection can present with metabolic disease by inducing insulin resistance. Intervirol; 49: 51-57.

[12]. Knober H, Stagnaro-Green A, Wallenstein S, Schwartz M, Roman SH (1998): Higher incidence of diabetes in liver transplant recipients with hepatitis C. J din Gastroenterol 1998; 26;30-33.

[13]. Knobler H, Schihmanter R and Zifroni A (2000): Increased risk of type 2 diabetes mellitus in non-cirrhotic patients with hepatitis C. Mayo ClinProc; 75:355–359.

[14]. Konrad T, Zeuzem S, Toffolo G, Vicini P, Teuber G, Briem D, Lormann J, Lenz T, Herrmann G, Berger A, Cobelli C and Usadel K (2000): Severity of HCV-induced liver damage alters glucose homeostasis in noncirrhotic patients with chronic HCV infection. Digestion, 62 , 52– 59.

[15]. Lecube A., Hernandez C and Simó R (2007): Glucoseabnormalities are an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. Am J Gastroenterol; 102: 2189-2195.

[16]. Maeno T, Okumura A, Ishikawa T, Kato K, Sakakibara F, Sato K, Ayada M, Hotta N, Tagaya T, Fukuzawa Y and Kakumu S (2003): Mechanisms of increased insulin resistance in non-cirrhotic patients with chronic hepatitis C virus infection. J Gastroenterol Hepatol, 18, 1358–1363.

[17]. Maggi G, Bottelli R and Gola D (1996): Serum cholesterol and chronic hepatitis C. Ital J Gastroenterol; 28:436–440.

[18]. Marchesini G, Bugianesi E and Forlani G (2003): Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology.; 37:917-923.

[19]. Massini M, Campani D, Boggi U, Menicagli M, Funel N, Pollera M, Lupi R, Del Guerra S, Bugliani M, Torri S, Del Prato S, Mosca F, Filipponi F and Marchetti P (2005): Hepatitis C virus infection and human pancreatic beta-cell dysfunction. Diabetes Care ,28, 940– 941.

[20]. Mason A and Nair S (2003): Is type II diabetes another extrahepatic manifestation of HCV infection? AM J Gastroenteral; 98:243-246.

[21]. Mason AL, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK and Perrillo RP (1999): Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology, 29 , 328– 333.

[22]. Mehta SH, Bran cati FL, Sulkowski MS, Strathdee SA and Thomal DL (2000):Prevelance of type 2 diabetesmellittes among persons with hepatitis C virus infection in united states. Ann Intern Med ; 133:592-599.

[23]. Monazahian M, Bohme I and Bonk S (1999): Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol; 57:223-229.

[24]. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T and Koike K (2003):Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol, 78 (Pt 7), 1527–1531.

[25]. Nicderau C, Lange S, Heintges T, Erhardl A, Buschkamp M and Hurter D (1998):Prognosis of chronic hepatitis C : results of a large, prospective chort study. Hepatology ; 28: 1687-1695.

[26]. Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Male PJ and Mentha G (2000): Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. J Hepatol ; 33:106-115.

[27]. Serfaty L, Andreani T and Giral P (2001): Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. J Hepatol; 34: 428-434.

[28]. Shaheen M, Echeverry D and Oblad MG (2007): Hepatitis C, metabolic syndrome, and inflammatory markers: results from the Third National Health and Nutrition Examination Survey [NHANES III]. Diabetes Res ClinPract.; 75:320–326.

[29]. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K and Koike K (2004): Hepatitis C virus infection and diabetes: Direct involvement of the virus in the development of insulin resistance. Gastroenterology, 126 , 840– 848.
[30]. Thomssen R, Bonk S and Thiele A. (1993):Density heterogeneities of hepatitis C virus in human sera due to the binding of Blipoproteins and immunoglobulins. Med MicrobiolImmunol; 182:329–334.
[31]. Trinder P (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann ClinBiochem.; 6: 24-27.
[32]. Yazicioglu F, Isitan H, Altunbas I, Sulemanlar M, Oxdogan MK, Balci U and Karayalcin (2004): Insulin resistance inchronic hepatitis C. Int J clinpract ; 58,11, 1020-1022 .