Insulin Resistance and Hyperleptinemia in Non-Diabetic Patients With Chronic Hepatitis C Virus Infection and Their Relations To Steatosis and Fibrosis Progression

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ABSTRACT

Chronic hepatitis C virus infection is associated with an increased prevalence of type 2 diabetes mellitus. The present work aimed to investigate the virus-induced insulin resistance in non diabetic patients as well as serum leptin and their relations to steatosis and fibrogenesis in chronic hepatitis C (CHC) infection. Twenty-Five patients, 15 males and 10 females with CHC were compared with 25 subjects, matched for age, sex and body mass index (BMI). The results of the work showed that the mean values of serum insulin, C-peptide, HOMA-IR (Homeostasis Model Assesment-Insulin Resistance) and leptin were significantly elevated compared with the control group ($p<0.05$). Also, HOMA-IR was significantly correlated with age, BMI, serum albumin, C-peptide, leptin and histopathological findings ($p<0.05$).

From the results of the present study, it could be concluded that hepatitis C virus (HCV) infection might be suggested as an additional risk factor for the development of hyperinsulinemia, insulin resistance, and hyperleptinemia that may be a mechanism for steatosis and fibrogenesis in patients with CHC. Continuous screening of these patients might be recommended for early detection and better management of impending diabetes mellitus.

INTRODUCTION

Acute hepatitis C develops into chronic hepatitis in more than 50% of patients ***(Kage et al.,1997).*** A proportion of patients with chronic hepatitis C (CHC) progress to cirrhosis and hepatocellular carcinoma ***(Yano et al.,1996).***

Recent evidence suggests that CHC infection is associated with an increased risk for the development of type 2 diabetes irrespective of whether cirrhosis is present ***(Shintani et al.,2004).***

It has been suggested that the connection between hepatitis C and diabetes mellitus (DM) could be secondary to the ability of HCV to induce hepatic steatosis ***(Patton et al.,2004).***

Insulin resistance (IR) plays a primary role in the development of type 2 diabetes mellitus. This is supported by prospective longitudinal and cross sectional studies showing that IR is the best predictor for the development of diabetes mellitus. It precedes the onset of diabetes 10-20 years. IR appeared important to determine whether HCV infection can predispose to the development of IR before diabetes occurs. Such potential link is particularly cogent in light of recent data which indicate that diabetes may be associated with an increased fibrosis progression in CHC ***(Monto et al.,2002).***

In addition, leptin may be considered a new endocrine mediator, beside its obvious role in body weight regulation. A possible interaction was suggested between leptin and insulin as the latter can regulate leptin expression, high serum leptin levels are an important aetiological factor of insulin resistance in patients with CHC infection (***Öncül et al.,2002a)***.

Liver fibrosis should now be added to the list of conditions in which leptin plays a crucial role. It enhances procollagen mRNA that reflects the synthesis of type I collagen in the liver. Also, leptin augments both proinflammatory and profibrogenic responses induced by hepatotoxic chemicals. It is postulated that the increase in systemic leptin levels

enhances up-regulation of transforming growth factor-β (TGF-β), leading to activation of hepatic stellate cells, thereby augmenting the fibrogenic response in the liver ***(Ikejima et al.,2001)***.

SUBJECTS AND METHODS

Twenty-Five patients from the Gastroenterology Department, Benha University Hospital were volunteers for the study. Their ages ranged from 22 to 60 years, (mean 46.4±11.3). They were 15 males and 10 females. These patients were diagnosed as having CHC infection. .Another, 25 healthy subjects with matched age, sex and BMI volunteered as controls.

All the patients and controls were subjected to the following investigations : medical history taking, general and local examination and ultrasonography scanning of the abdomen. BMI was determined according to ***Garrow,1990***. Laboratory investigations including fasting serum glucose ***(Trinder,1969),*** cholesterol ***(Stein,1986)*** , triglycerides ***(Wahlfeld,1974),*** liver function tests including serum bilirubin ***(Malloy and Evelyn,1937),*** aspartate aminotransferase (AST) ***(Reitman and Frankel,1957),***alanine aminotransferase (ALT) ***(Reitman and Frankel,1957),*** alkaline phosphatase ***(Teitz and Shuey,1986)***, γ-glutamyl transferase (GGT) ***(Szasz et al.,1974)***, total protein ***(Bakerman,1984)*** and Albumin ***(Dumas et al.,1971)***, hepatitis markers as hepatitis B surface antigen (HBsAg) by immuno-chromotographic analysis ***(Blumberg,1971)***, hepatitis C virus antibodies (HCVab) by immuno-chromotographic analysis ***(Arash et al.,1993)*** and qualitative HCV (RNA) PCR for patients who were reactive with HCVab ***(Hitzler and Runkel,2001)***, serum insulin by radio-immunoassay (RIA) ***(Robinson et al.,1996)***, C-peptide by RIA ***(Myrick et al.,1989) ,*** HOMA-IR which was calculated:

Fasting serum insulin (μIU/ml) X Fasting serum glucose (mmol/l) / 22.5 ***(Emoto et al.,1999),*** leptin by ELISA ***(Friedman and Halaas,1998)*** and histopathological examination of liver biopsy for steatosis as well as fibrogenesis grading and staging ***(The French METAVIR cooperative study group,1994)*** were done for these patients.

Exclusion criteria:

Patients with hepatic decompensation (Hepatic encephalopathy, ascites, variceal bleeding or serum total bilirubin >2.0 mg/dl), diabetics, concurrent active hepatitis B virus, HIV patients, autoimmune hepatitis, hemochromatosis, or Wilson disease.

Sampling:

Venous blood sample (7.0 ml) was taken from all volunteers after overnight fasting. The sample was divided into 2 parts. The first part (2.0 ml) was taken in a sterile tube containing EDTA solution for qualitative determination of HCV (RNA) by PCR. The remaining part (5.0 ml) was left to clot, centrifuged and the serum separated was used for determination of glucose, total cholesterol, tiglycerides, bilirubin, AST and ALT, alkaline phosphatase, γ-glutamyl transferase, total protein, Albumin, HBsAg, and HCVab . The remaining part of the serum was kept frozen at – 80^{o}C for later determination of serum insulin, C-peptide level and leptin.

Histological studies and steatosis evaluation:

Liver biospy specimens of more than 10 mm in length were fixed in formalin, paraffin embedded, and stained with haematoxylin-eosin-safran, or picroSirius red for collagen, and Perls' technique for iron. For each liver biopsy specimen, histological fibrosis and activity were scored according to the METAVIR classification. Steatosis was graded as follows: none; mild (involving less than 10% of hepatocytes); moderate

(involving 10–30% of hepatocytes); and severe (involving more than 30% of hepatocytes).

Grading of histological activity that evaluates the intensity of necroinflammatory lesions was as follows: A0, no activity; A1, mild activity; A2, moderate activity; and A3, severe activity. Fibrosis was staged on a 0–4 scale: F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis.

Statistical analysis:

The results of the work were tabulated and statistically analyzed using student unpaired t- test and Pearson's bivariate correlation. p values more than 0.05 were considered insignificant, while p values of 0.05 or less were considered significant ***(Budneck,1987).***

RESULTS

Table (1) shows the demographic distribution of cases according to histopathological picture.

Fig(1) shows severe liver steatosis while Fig.(2) shows liver fibrosis grade A2,stage F3.

Table (2) shows mean ±SEM and p values of age, BMI, serum glucose, total cholesterol, triglycerides, liver function tests in CHC compared to the control group.

Table (3) shows that the mean value of serum insulin (25.67 ±3.03 µIU/ml vs 11.44 ±0.94 µIU/ml), C-peptide (6.07±1.05 µIU/ml vs 1.65 ±0.28 µIU/ml), HOMA-IR (6.14 ±0.63 vs 2.65 ±0.18) and leptin (13.77 ±0.42 ng/ml vs 6.20±0.29 ng/ml) were significantly elevated in CHC virus group compared with the control group ($p<0.05$ for each).

Table (4) shows that HOMA-IR was significantly positive correlated with age ($r=0.47;p<0.05$), BMI ($r=0.41;p<0.05$), C-peptide ($r=0.50;p<0.05$), leptin ($r=0.42;p<0.05$) and histopathological steatosis ($r=0.41;p<0.05$), fibrosis grading ($r=0.70;p<0.05$), Staging

(r=0.48;p<0.05), and negative correlation with serum albumin (r=-0.46;p<0.05).

Table (1): Demographic distribution of cases according to histopathological picture.

Histopathology	CHC group (n=24)
Steatosis:	
Mild:	**12 (50%)**
Moderate:	**10 (41.7%)**
Severe:	**2 (8.3%)**
Grading of fibrosis:	
A0:	**0 (0%)**
A1:	**8 (33.3%)**
A2:	**12 (50%)**
A3:	**4 (16.7%)**
Staging of fibrosis:	
F0:	**0 (0%)**
F1:	**10 (41.7%)**
F2:	**8 (33.3%)**
F3:	**6 (25%)**
F4:	**0 (0%)**

Fig(1):severe liver steatosis

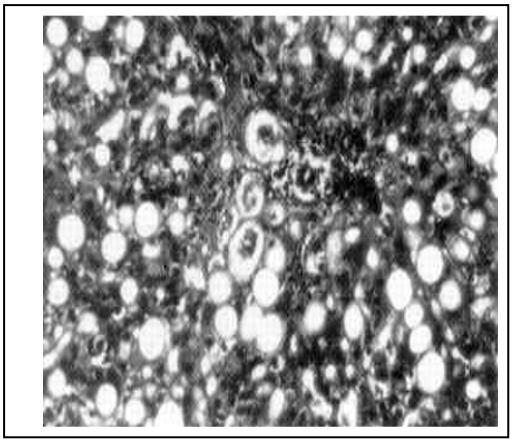

Fig.(2):Liver fibrosis grade A2,stage F3

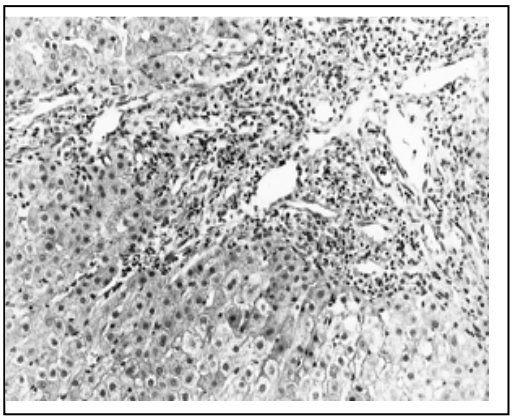

Table (2): mean and ±SEM of age, BMI, serum fasting glucose, total cholesterol, triglycerides, liver function tests in the studied groups.

Groups / Biochemical Parameters	Controls (n = 25)	CHC group (n = 25)	p
Age (Y)	40.68 ±2.52	46.48 ±2.27	N.S
Body mass index (Kg/m^2)	23.60 ±0.22	23.80 ±0.28	N.S
Serum fasting glucose (mmol/l)	5.49 ±0.22	5.64 ±0.20	N.S
Serum total cholesterol (mmol/l)	3.77 ±0.13	4.54 ±0.18	<0.05
Serum triglycerides (mmol/l)	1.22 ±0.10	1.27 ±0.14	N.S
S.Total Bilirubin (mg/dl)	0.63 ±0.04	1.68±0.03	<0.05
S.Direct Bilirubin (mg/dl)	0.16 ±0.01	0.44±0.02	<0.05
S.Indirect Bilirubin(mg/dl)	0.47 ±0.04	1.24±0.04	<0.05
S.AST (U/l)	20.88 ±1.32	84.48 ±3.16	<0.05
S.ALT (U/l)	35.76 ±0.81	68.28 ±4.26	<0.05
S.Alkaline Phosphatase (U/l)	77.08 ±3.11	174.04 ±6.53	<0.05
SGGT(U/l)	32.24 ±2.06	55.44 ±4.11	<0.05
S.Total Protein(g/l)	76.32 ±1.05	71.68 ±1.52	<0.05
S.Albumin(g/l)	39.84±0.92	34.44 ±1.23	<0.05
S.Globulin(g/dl)	36.48 ±0.96	37.24±1.07	N.S

p>0.05: non-significant (N.S).
p<0.05: significant.

Table (3): mean, ±SEM and p values of serum serum insulin, C-peptide, HOMA-IR and leptin in patients with CHC compared with the control group.

Groups / Biochemical Parameters	Controls (n = 25)	CHC group (n=25))	p
S. insulin (µIU/ml)	11.44 ±0.94	25.67 ±3.03	<0.05
S. C-peptide (µIU/ml)	1.65 ±0.28	6.07 ±1.05	<0.05
HOMA-IR	2.65 ±0.18	6.14 ±0.63	<0.05
S.leptin (ng/ml)	6.20 ±0.29	13.77 ±0.42	<0.05

p<0.05: significant.

Table (4): correlation coefficient (r) between HOMA-IR, age, BMI,S.cholesterol, S.triglycerides, liver function tests, S. C-peptide and liver biopsy in patients with HCV liver cirrhosis.

Group / Biochemical Parameters	CHC group (n=25)	
	(r)	(p)
Age (Y)	0.47	p<0.05
Body mass index (BMI) Kg/m^2	0.40	p<0.05
Serum total cholesterol(mmol/l)	0.21	N.S
Serum triglycerides (mmol/l)	0.09	N.S
Serum total bilirubin (mg/dl)	0.21	N.S
Serum direct bilirubin (mg/dl)	0.22	N.S
S.Indirect Bilirubin (mg/dl)	0.05	N.S
Serum AST (U/l)	0.003	N.S
Serum ALT (U/l)	0.06	N.S
Serum alkaline phosphatase (U/l)	0.05	N.S
SGGT(U/l)	0.36	N.S
Serum total protein (gm/dl)	-0.37	N.S
Serum albumin (gm/dl)	-0.46	p<0.05
Serum globulin (gm/dl)	0.004	N.S
Serum C-peptide (µIU/ml)	0.50	p<0.05
Serum leptin (ng/ml)	0.42	p<0.05
Liver pathology:		
Steatosis :	0.41	p<0.05
Fibrosis grading :	0.70	p<0.05
Fibrosis staging :	0.48	p<0.05

p>0.05: non-significant (N.S). p<0.05: significant.

DISCUSSION

Among patients infected with HCV,13-33% develop type 2 diabetes mellitus. The mechanism for this remains unclear ***(Knobler et al.,2003).***

The present work showed that HOMA-IR, serum insulin, C-peptide and leptin were significantly elevated in non-diabetic patients without cirrhosis infected with HCV compared to the control group (p<0.05).

The association between HOMA-IR and CHC were hypothized by different authors. The overall mechanisms include:

First, the peripheral hyperinsulinemia observed in subjects with chronic hepatic disease could be attributed to diminished insulin removal by the diseased liver rather than pancreatic hypersecretion ***(Bonora et al.,1984a)***. Thus, hyperinsulinemia and high concentrations of counter regulatory substances might play a role in the pathogenesis of insulin resistance in subjects suffering from chronic liver disease ***(Bonora et al.,1984b)***.

Second, serum HCV core protein was reported to down-regulate the expression of hepatic insulin receptor substrate-1 (IRS-1) and IRS-2. However, disruption of IRS-1 results in insulin resistance but not DM, because of compensatory hyperinsulinemia while disruption of IRS-2 results in severe DM because of insulin resistance and disturbances of insulin secretion ***(Kawaguchi et al.,2004)***.

Third, tumor necrosis factor-α (TNF-α) which is a pro-inflammatory cytokine was found to be significantly elevated in patients with CHC. TNF-α could induce serine phosphorylation of IRS-1 and thus inhibits its tyrosine phosphorylation and signaling activity ***(Hotamisligil,1999)*** **.** This defects in insulin receptors will lead to hyperinsulinemia ***(Aytug et al.,2003)***. Also, TNF-α can induce steatohepatitis and insulin resistance might be a consequence of steatosis.

Fourth, another possible explanation is a direct effect of HCV proteins on insulin signaling pathways ***(Previs et al.,2000).***

Fifth, serum ferritin which represents an acute phase reactant protein was reported to be elevated in patients with CHC. Excess iron is usually stored in the liver, muscle and pancreas and may cause organ-specific oxidative damage leading to insulin resistance and eventually beta- cell failure ***(Larson et al.,2003).***

Finally, high serum leptin levels cause desensitization of the receptor and thus defective leptin receptor signaling in β-cells which leads to chronic hyperinsulinemia and may thus contribute to the

pathogenesis of DM. Also, leptin receptors are present on human hepatocytes, and leptin was shown to modulate several insulin-induced activities in these cells. Leptin antagonizes insulin signaling by decreasing insulin-induced tyrosine phosphorylation of IRS-1. It increases phosphoenol pyruvate carboxykinase (PEPCK) and decreases glucokinase expression, leading to increased gluconeogenesis. The hepatic effects of leptin levels may thus contribute to hepatic insulin resistance ***(Fukui et al.,2002).***

The results of the present work were in accordance with the findings of ***Testa et al.,(2000), Öncül et al.,(2002a) Maeno et al.,(2003)*** and ***Hui et al.,(2003).***

Furthermore, the current work showed that HOMA-IR had significant positive correlation with age, BMI, serum insulin, C-peptide, leptin, histopathological steatosis, grading and staging of fibrosis and inversely correlated with serum albumin ($p<0.05$).

Steatosis can either originate from insulin resistance resulting in metabolic steatosis or from a direct cytopathic effect of the virus resulting in viral steatosis ***(Ratziu et al.,2004).***

Insulin resistance allows for enhanced lipolysis and the generation of free fatty acids (FFAs) for hepatic reesterification and oxidation. The accumulation of fat in the liver increases its vulnerability to various secondary insults, which cause necroinflammatory changes, steatosis, fibrosis and eventually cirrhosis ***(Nanji,2004)***. Exaggerated levels of FFAs may be deleterious for the liver through a variety of mechanisms including de novo synthesis of ceramides which may cause apoptosis, resistance to insulin by interfering with intracellular phosphorylation processes and lipid peroxidation as a consequence of the increased production of free radicals. These free radicals can induce Fas (FS-7 associated cell surface antigen) - ligand expression in hepatocellular membranes since its promoter contains a binding site for nuclear factor

kappa-B (NfκB). Interaction of Fas ligand with Fas-expressing hepatocytes leads to their death through a process termed "Fratricidal apoptosis" ***(Medina et al.,2004)***. The ingestion of apoptotic bodies by stellate cells stimulates fibrogenesis which is further activated by lipid peroxidation and high leptin levels ***(Pessayre et al.,2004)***.

Moreover, hyperinsulinemia can directly stimulate hepatic stellate cells to proliferate and to secrete extracellular matrix proteins. Also, hyperinsulinemia causes upregulation of connective tissue growth factor, a cytokine involved in the pathogenesis of fibrosing liver diseases ***(Hui et al.,2003)***

Furthermore, viral steatosis may be due to the direct effect of HCV core gene on liver cells which is characteristic of CHC infection ***(Alexander,2000)*** Hepatic steatosis leads to an increase in lipid peroxidation in hepatocytes which in turn activates hepatic stellate cells. These cells produce transforming growth factor- β (TGF-β) which is a potent fibrogenic cytokine ***(Shimizu,2001).***

Moreover, leptin plays an important role in liver fat storage. Steatosis is a common finding in CHC infection. Overaccumulation of lipids in non-adipose tissues may lead to lipotoxic complications such as diabetes ***(Öncül et al.,2002b).*** However, hyperleptinemia levels were reported to be significantly higher in patients with steatosis and steatohepatitis. Also, leptin was reported to be expressed by hepatic stellate cells. Activation of these cells may lead to hyperleptinemia. Elevated serum leptin levels behave as a profibrogenic cytokine causing overexpression of smooth muscle actin and TGF-β, a potent profibrogenic cytokine. These mediators lead to hepatic fibrosis ***(Marra,2002)***.

Thus, steatosis reflects an interaction of viral and host factors important in the generation of fibrosis in the liver. It seems that steatosis

and fibrosis was found to be strongly associated in non-cirrhotic liver and once present tend to persist ***(Wyatt et al.,2004).***

The results of the present work were compatible with the findings of ***Petit et al.,(2001) and Hui et al.,(2003)*** who reported that HOMA-IR was significantly correlated with age, BMI, albumin, C-peptide and grading of liver fibrosis.

It could be concluded that hepatitis C virus (HCV) infection is suggested as an additional risk factor for the development of hyperinsulinemia and insulin resistance that may be a mechanism for steatosis and fibrogenesis in patients with CHC. Continuous screening of these patients is recommended for early detection and better management of impending diabetes mellitus.

REFERENCES

Alexander,G.J.M.(2000)

An association between hepatitis C virus infection and type 2 diabetes mellitus: what is the connection?
Ann. Intern. Med., 133: 650.

Arash,G.,Czeslaw,W.,Lin ,C.,Feinstone,S.M. and Rice,C.M.(1993)

Expression and identification of hepatitis C virus polyprotein cleavage products.
J . Virology P:1385.

Aytug, S., Reich, D., Sapiro, L.E., Bernstein, D. and Begum, N. (2003)

Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology 38(6): 1384.

Bakerman,A.S.(1984)

Determination of total protein.
In: ABC,s of interpretative laboratory data, 2nd edition, Griffin and Tilghman,PP:374.

Blumberg,B.S.(1971)

The discovery of Australian antigen and its relation to viral hepatitis.
Vitro 7:223.

Bonora, E., Coscelli, C ., Orioli, S ., Cambi, R .,Buzzelli, G., Gentilini, P. and Butturini, U.(1984a)

Hyperinsulinemia of chronic active hepatitis: impaired insulin removal rather than pancreatic hypersecretion.
Horm. Metab. Res., 16(3): 111.

Bonora, E ., Orioli, S., Coscelli, C., Buzzelli, G., Gentilini, P. and Butturini, U.(1984b)
Possible roles of insulin, glucagon, growth hormone and free fatty acids in the pathogenesis of insulin resistance of subjects with chronic liver diseases.
Acta Diabetol. Lat., 21(3): 241.

Budneck, L. (1987)
Statistics" in: Cassem, B.J: preventive medicine and public health. PP. 43-77. New York, John Wiley and Sons.

Dumas,B.T,Watson,W.A. and Biggs,H.G. (1971)
Quantitative colorimetric determination of albumin in serum or plasma. Clin.Chim.Acta 31:87.

Emoto, M., Nishizawa, Y., Maekawa, K., Hiura, Y., Kanda, H., Kawagishi, T., Shoji, T., Okuno, Y. and Morii , H. (1999)
Homeostasis model assessment as a clinical index of insulin resistance in type 2 diabetic patients treated with sulfonylureas Diabetes Care 22: 818.

Friedman J.M. and Halaas, J.L. (1998)
Leptin and the regulation of body weight in mammals.
Nature 395(6704): 763.

Fukui, M., Kitagawa, Y., Nakamura, N. and Yoshikawa, T. (2002)
Response to Öncül: Insulin sensitivity in patients with chronic hepatitis C virus infection
Diabetes Care 25: 1900.

Garrow, J. (1990)
Is it possible to prevent obesity?
Infusions therapie 17: 28.

Hitzler,W.E. and Runkel, S . (2001)
Routine HCV PCR screening of blood donations to identify early HCV infection in blood donors lacking antibodies to HCV.
Transfusion 41(3):333.

Hotamisligil,G.S.(1999)
The role of TNFalpha and TNF receptors in obesity and insulin resistance.
J. Intern. Med., 245(6): 621.

Hui, J.M., Sud, A., Farrell, G.C., Bandara, P., Byth, K., Kench, J.G., McCaughan, G.W. and George, J. (2003)
Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. Gastroenterology 125(6): 1695.

Ikejima, K., Honda, H., Yoshikawa, M., Hirose, M., Kitamura, T., Takei, Y., and Sato, N. (2001)
Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals.
Hepatology 34(2): 288.

***Kage, M., Shimamatu, K., Nakashima, E., Kojiro, M., Inoue, O. and Yano,M.*(1997)**
Long-term evolution of fibrosis from chronic hepatitis to cirrhosis in patients with hepatitis C: morphometric analysis of repeated biopsies.
Hepatology 25(4): 1028.

Kawaguchi, T., Yoshida, T., Harada, M., Hisamoto, T., Nagao, Y., Ide, T., Taniguchi, E., Kumemura, H., Hanada, S., Maeyama, M., Baba, S., Koga, H., Kumashiro, R., Ueno, T., Ogata, H., Yoshimura,A. and Sata,M .(2004)
Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3
Am. J. Pathol., 165: 1499.

Knobler, H ., Zhornicky, T., Sandler, A ., Haran, N., Ashur, Y .and Schattner, A.(2003)
Tumor necrosis factor-alpha-induced insulin resistance may mediate the hepatitis C virus-diabetes association.
Am. J. Gastroenterol., 98(12): 2751.

Larson, A.M., Taylor, S.L., Bauermeister, D., Rosoff, L. and K.V. Kowdley,J.R.(2003)
Pilot study of the relationship between histologic progression and hepatic iron concentration in chronic hepatitis C.
J. Clin. Gastroenterol., 37(5): 406.

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(12): 1358.

Malloy,H.T. and Evelyn, K.A (1937)
Quantitative determination of direct bilirubin and total bilirubin in serum , heparinized plasma or EDTA plasma.
J.Biol.Chem.,119:481.

Marra, F.(2002)
Leptin and liver fibrosis: a matter of fat.
Gastroenterology 122(5): 1529.

Medina, J., Fernández-Salazar, L. I., García-Buey, L. and Moreno-Otero,R.(2004)
Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis
Diabetes Care 27: 2057.

Monto,A., Alonzo, J., Watson, J.J., Grunfeld, C. and Wright, T.L.(2002)
Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus and alcohol.
Hepatology 36(3): 729.

Myrick, J.E., Gunter, E.W., Maggio, V.L., Miller, D.T. and Hannon, W.H.(1989)
An improved radioimmunoassay of C-peptide and its application in a multiyear study
Clin. Chem., 35: 37.

Nanji,A.A.(2004)
Another animal model for nonalcoholic steatohepatitis: how close to the human condition?
Am. J. Clin. Nutr., 79: 350 .

Öncül, O., Top, C., and Cavuslu, S. (2002a)
Response to Fukui et al.
Diabetes Care 25: 1901.

Öncül, O., Top, C. and Çavuþlu, Þ.(2002b)
Correlation of serum leptin levels with insulin sensitivity in patients with chronic hepatitis-C infection
Diabetes Care 25: 937.

Patton, H.M., Patel, K., Behling, C., Bylund, D., Blatt, L.M., Vallee, M., Heaton, S., Conrad, A., Pockros, P.J. and McHutchison,J.G.(2004)
The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. J. Hepatol., 40(3): 484.

Pessayre, D., Fromenty, B. and Mansouri, A. (2004)
Mitochondrial injury in steatohepatitis.
Eur. J. Gastroenterol. Hepatol., 16(11): 1095.

Petit J.M.,, Bour, J.B., Galland-Jos, C., Minello, A., Verges, B., Guiguet, M., Brun, J.M. and Hillon, P. (2001)
Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C.
J. Hepatol., 35(2): 279.

Previs, S. F., Withers, D. J., Ren, J., White, M. F. and Shulman, G. I. (2000)
Contrasting effects of IRS-1 *Versus* IRS-2 gene disruption on carbohydrate and lipid metabolism *in Vivo*
J. Biol. Chem., 275: 38990.

Ratziu, V., Trabut, J.B. and Poynard, T. (2004)
Fat, diabetes, and liver injury in chronic hepatitis C. Curr. Gastroenterol. Rep., 6(1): 22.

Reitman, S. and Frankel, S. (1957)
A colorimetric method for determination of glutamic- oxaloacetate and glutamic – pyruvate transaminases.
Ann. J. Clin. Path., 28:56.

Robinson,.C.,Anderson,L. and Bowsher,R.(1996)
"Standardization of insulin assay" Report of the American Diabetes Association's Task Force,42(2):242.

Shimizu,I.,(2001)
Antifibrogenic therapies in chronic HCV infection. Curr. Drug. Targets. Infect. Disord., 1(2): 227.

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(3): 840.

Stein, E. A. (1986):
Determination of total cholesterol by enzymatic method.
In : Text book of clinical chemistry. Tietz, N.W. (ed.) W.B Saunders, Philadelphia,PP: 879.

Szasz,G.,Persijn,J.P.Y. and Cols,Z.(1974)
Kinetic determination of γ–glutamyl transferase activity.
Clin.Chem.Clin.Biochem.,12:228.

Teitz,N.W. and Shuey,D.F.(1986)
Kinetic determination of alkaline phosphatase activity.
Clin.Chem.,32:1593.

Testa, R., Franceschini, R., Giannini, E., Cataldi, A., Botta, F., Fasoli, A., Tenerelli, P., Rolandi, E. and Barreca, T. (2000)
Serum leptin levels in patients with viral chronic hepatitis or liver cirrhosis.
J. Hepatol., 33(1): 33.

The French METAVIR Cooperative Study Group (1994)
Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C.
Hepatology 20:15.

Trinder, P. (1969).
Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor.
Ann. Clin. Biochem., 6 : 24.

Wahlefeld, A.W. (1974).

Quantitative enzymatic determination of triglycerides in serum or plasma.
In : methods of enzymatic analysis vol. (5), Bergineyer hu (ed.). academic press, N.Y, PP : 1831.

Wyatt, J., Baker, H., Prasad, P., Gong, Y. Y. and Millson , C., (2004)

Steatosis and fibrosis in patients with chronic hepatitis C
J. Clin. Pathol., 57: 402.

Yano, M., Kumada, H., Kage, M., Ikeda, K., Shimamatsu, K., Inoue, O., Hashimoto, E., Lefkowitch, J.H., Ludwig, J. and Okuda,K.(1996)

The long-term pathological evolution of chronic hepatitis C. Hepatology 23(6): 1334.

الملخص العربى

مقاومة الإنسولين و زيادة مستوى اللبتين بمصل الدم فى مرضى الإلتهاب الكبدى الفيروسى"سى" المزمن والغير مصابين بمرض البوال السكرى و علاقتهما بمدى تكون الشحوم والألياف بالكبد

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لوحظ وجود زيادة فى معدل الإصابة بمرض البوال السكرى من النوع الثانى بين المرضى المصابين بالإلتهاب الكبدى الفيروسى"سى". يهدف هذا البحث إلى دراسة مقاومة الإنسولين و زيادة مستوى اللبتين بمصل الدم فى مرضى الإلتهاب الكبدى الفيروسى"سى" المزمن والغير مصابين بمرض البوال السكرى وتصلب الكبد و علاقتهما بمدى تكون الشحوم والألياف بالكبد. تم إجراء هذه الدراسة على 50 شخصا من المتطوعين من الجنسين ممن تتراوح أعمارهم 22-60 سنة. وقد قسموا إلى مجموعتين: شملت الأولى على 25 شخصا من الأصحاء كمجموعة ضابطة والثانية على 25 مريضا يعانون من مرض الإلتهاب الكبدى الفيروسى"سى" المزمن. تشير نتائج هذا البحث إلى وجود زيادة ذات دلاله إحصائيه فى مستوى الإنسولين والببتيدات"سى" و مقاومة الإنسولين و اللبتين بمصل الدم فى مرضى الإلتهاب الكبدى الفيروسى"سى" المزمن عند مقارنتهم بالمجموعه الضابطه. وكذلك وجد أن هناك علاقة إرتباط طردية و ذات دلالة إحصائية بين مقاومة الإنسولين

وعمرالمرضى ومعامل السمنة ومستوى الببتيدات"سى" و اللبتين بمصل الدم و درجة تشحم و تليف الكبد بينما أن هذه العلاقة عكسية مع مستوى الزلال بمصل الدم.

هذا البحث يوضح أن إلتهاب الكبدى الفيروسى"سى" المزمن يشكل خطوره إضـــافية لهؤلاء المرضى من حيث زيادة مستوى مقاومة الإنسولين و اللبتين بمصل الدم ممـــا قـــد يسبب زيادة تشحم و تليف الكبد. ولذلك توصى هذه الدراسة بمباشرة هؤلاء المرضى بصفة مستمرة لإكتشاف مرض البوال السكرى وعلاجه مبكرا.