Diabetes Mellitus and peripheral Insulin Resistance in Egyptian Chronic HCV Patients Treated with Standard Antiviral Therapy

Amal S. Bakir, Kadry M Elsaeed, Marcel W. Keddeas

Faculty of Medicine, Ain Shams University, Cairo, Egypt.
amalshawky_mb@hotmail.com

Abstract :Background : Patients with hepatitis C virus (HCV) infection present higher risk of developing type-2 diabetes mellitus (DM). However, the mechanism of this association and the role of antiviral treatment are still unclear**. Aim**: To study the prevalence of insulin resistance & DM in chronic hepatitis C patients, whether received treatment or not, and the effect of treatment. **Subjects and Methods:** This study was conducted on 75 subjects who were divided into three groups; *Group A*: 15 non diabetic non HCV subjects as a control group. *Group B*: 30 patients with chronic hepatitis C but still untreated, and *Group C*: 30 patients with chronic hepatitis C treated by standard doses of pegylated interferon plus ribavirin. Routine laboratory investigations, fasting insulin level, Homeostasis model assessment – estimated insulin resistance (HOMA-IR), HCV- Ab., HCV-RNA, and liver biopsy were done. **Results**: Untreated chronic HCV patients (group B) demonstrated significant changes in HbA1c, and highly significant changes in post prandial blood sugar(PPBS), fasting insulin level, and HOMA-IR after 6 months. These changes were associated with decrease in insulin sensitivity from 73.33% to 36.67%, whereas insulin resistance increased from 26.67% to 46.67%, in addition to appearance of five cases of newly diagnosed DM (16.7%), and these findings were highly significant. On the other hand, treated chronic HCV patients (group C) showed a highly significant changes as regards AST and ALT, and non significant changes in HbA1c, fasting insulin level, and HOMA-IR. Insulin sensitivity at beginning of the study was (53.33 %) and after 6 months increased by (13.33 %) ,whereas insulin resistance at beginning of the study was (46.67 %) and after 6 months increased by (23.33 %) and diabetes at beginning of the study was (0 %) and after 6 months was (10 %), and all were statistically insignificant. On correlating insulin resistance state with virologic response ,which was 40% at the end of the study, we noticed a significant increase in insulin resistance in chronic HCV patients who did not respond to treatment,(16 out of 18 patients showed insulin resistance), while 11 out of the 12 patients who turned HCV negative showed insulin sensitivity. **Conclusion:** chronic hepatitis C virus infection may be considered as a risk factor for development of insulin resistance and DM. Viral eradication is associated with improved insulin sensitivity. We recommend close and long term monitoring of non-responder chronic HCV patients for potential increased risk of developing DM.
[Amal S. Bakir, Kadry M Elsaeed, Marcel W. Keddeas. **Diabetes Mellitus and peripheral Insulin Resistance in Egyptian Chronic HCV Patients Treated with Standard Antiviral Therapy**. *Journal of American Science*. 2012;8(4): 814-818]. (ISSN: 1545-1003). http://www.americanscience.org. 109

Keywords: Diabetes Mellitus, insulin resistance, hepatitis c virus.

1. Introduction

Patients with hepatitis C virus (HCV) infection present higher risk of developing type-2 diabetes mellitus (DM). However, the mechanism of this association and the role of antiviral treatment are still unclear [1].

A large number of clues have suggested the potential role of a common hepatotrophic virus in developing diabetes. As many as 80% of patients with cirrhosis show glucose intolerance, and 10–20% of them have DM [2].

Insulin resistance (IR) is known to be associated with the visceral adipose tissue area. Elucidation of the relationship between hepatitis C virus (HCV) and IR is of great clinical relevance, because IR promotes liver fibrosis [3].

So, the aim of this work is to evaluate peripheral insulin resistance(IR) and prevalence of diabetes mellitus in patients with chronic hepatitis C before treatment and after 24-weeks therapy with pegylated interferon plus ribavirin.

2. Subjects and Methods:

2.1 Subjects: This study included 60 patients with chronic hepatitis C (group B & C). They were recruited from outpatient clinic of Ain Shams University hospitals, Cairo, Egypt. Group B included 30 patients of chronic hepatitis C but still untreated, whereas group C included 30 patients of chronic hepatitis C treated with pegylated interferon plus ribavirin. A control group (group A) included 15 healthy age and sex matched subjects was selected for comparison.

2.1.1. Inclusion criteria: Age between 20and 60 years, positive HCV by PCR, no use of antiviral medication for the last 6 months, histological evidence of necroinflammation and fibrosis evaluated by METAVIR score.

2.1.2. Exclusion criteria: HBV infection, patients with cirrhotic liver, chronic renal diseases, diabetes mellitus, and obesity (BMI $\geq$ 30).

Written informed consent was obtained from all subjects upon enrollment in the study and the study was approved by the local ethical committee.

2.2 Methods: All subjects were evaluated at the beginning of the study and six months later. In the first clinical evaluation, information about gender, race, blood transfusion, suspected time of infection, age, symptoms, previous use of antiviral medications, and if the patient had ever used interferon, personal and family history of arterial hypertension, obesity and dyslipideamia.

Physical examination was performed with special attention for blood pressure, body mass index (BMI), and waist circumference.

BMI was calculated by Quetelet index: Weight in Kg/ Height in meters squared (m^2).

Waist circumference was measured with a non-elastic measuring tape, in centimeters. Liver biopsy was done to evaluate degree of inflammation, steatosis and fibrosis index.

2.3. Laboratory analysis: The following laboratory tests were performed : Fasting and postprandial blood sugar (PPBS), HBA1c. Serum Insulin level, S. Creatinine, Na, K, CBC, ESR, complete lipid profile ,ALT, AST, HBsAg, HCV Ab., S. Albumin , PT,& PTT.

Real time quantitative PCR for all patients with chronic hepatitis C.

2.3.1. Abdominal Ultrasound (U/S): was done for all patients and control group.

Insulin resistance was calculated through the HOMA method as follows:

2.3.2. HOMA = fasting serum insulin (µu/ml) X serum glucose (mmol/dL) / 22.5.

Patients were categorized as insulin resistant if HOMA was greater than 2 and diabetes if greater than 4 [4].

2.3.3. Histopathological examination: Liver biopsy was performed for all patients of chronic hepatitis C at the beginning of the study .The degree of necro-inflammatory activity , fibrosis, and steatosis in the liver biopsy specimens were scored as described by Ishak et al., 1995 [5].

2.4. Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 17.0. Quantitative data were expressed as mean ± stander deviation (SD). Qualitative data were expressed as frequency and percentage. The following tests were applied Independent Samples T test, Chi-square test, pearson's correlation coefficient and Mann-whitney test

3. Results:

In this follow up study the prevalence of insulin resistance & DM in chronic hepatitis C patients, whether received treatment or not, was plotted against a control group of non diabetic patients without chronic hepatitis C.

This study was conducted on 75 subjects who were divided into three groups:

Group A: 15 subjects as a control group. They were 12 males (80%) & 3 females (20%) with their ages between (38-52) years. No significant changes could be demonstrated in the control group at beginning of the study and after 6 months as regard their demographic and laboratory data including PPBS, fasting insulin level, and HOMA-IR.

Group B: 30 patients with chronic hepatitis C but still untreated (refuse treatment) . They were 22 males (73%) and 8 females (27%) with their ages between (39-56) years. On follow up of those Untreated Chronic HCV Patients, no significant changes could be demonstrated as regards BMI ,waist circumference ,AST,ALT,PT, serum albumin , FBS , and lipid profile, whereas significant increase in HbA1c, and highly significant increase in PPBS, fasting insulin level, and HOMA-IR were seen after 6 months. Twenty two patients (73.3%) were insulin sensitive at the beginning of study, but this percentage decreased to 36.6 % after 6 months, whereas the prevalence of insulin resistance increased from 26.6% to 46.6% after 6 months ,in addition to appearance of five cases of newly diagnosed DM, and these findings were highly significant (Table1).

Group C: 30 patients with chronic hepatitis C treated by standard doses of pegylated interferon plus ribavirin. They were 18 males (60 %) & 12 females (40%) with their ages between (38-55) years. Virological response (HCV PCR –ve) was obtained in 12 patients (40 %) after 6 months of combined peg-interferon plus ribavirin therapy.

Regarding data at beginning of the study compared to data 6 months later, No significant changes could be demonstrated as regards BMI, waist circumference, s. albumin, PT, lipid profile, FBS, PPBS, HbA1c, fasting insulin level, and HOMA-IR, whereas a highly significant decrease in AST and ALT occurred.

The percentage of subjects showing insulin sensitivity at beginning of the study was (53.33 %) and after 6 months increased by (13.33 %) ,whereas insulin resistance at beginning of the study was (46.67 %) and after 6 months increased by (23.33 %) and diabetes at beginning of the study was (0 %) and after 6 months was (10 %), and all were statistically insignificant (Table2). But on correlating insulin resistance state with virologic response , we found a highly significant increase in insulin resistance in HCV patients who did not respond to treatment (16 out of 18 patients showed insulin resistance). On the other hand ,11 out of the 12 patients who turned HCV negative showed insulin sensitivity (Table 3). So regarding treated chronic HCV patients, non significant changes in HOMA-IR after 6 months of treatment is explained by the number of patients who developed insulin resistance in non responder was nearly the same as number of patients developed insulin sensitivity after eradication of HCV.

When we compared HOMA-IR with study parameters, we did not find correlation between HOMA-IR with age, serum albumin , PT, and HbA1c. On contrast, a significant correlation was seen with BMI and waist circumference, and a highly significant correlation with AST,ALT, cholesterol, postprandial blood sugar, HCV, and liver biopsy indices(inflammatory , steatosis , and fibrosis index.). Compared to control group, insulin resistance is significantly higher in HCV patients and it is improved with successful antiviral therapy.

Table (1): Percentage of subjects showing insulin sensitivity, insulin resistance and DM at the beginning of study and after 6 months in group B.

Variable	No. of subjects	Percentage
Insulin sensitivity	22	73.33%
After6 months	11	36.67%
Insulin resistant	8	26.67%
After 6 months new IR	14	46.67%
DM	0	0%
After 6 months new DM	5	16.7%
U	273.00	
p	< 0.01	
Sig.	HS	

Table (2): Percentage of subjects showing insulin sensitivity, insulin resistance and diabetes before and after treatment in group C

Variable		No. of subjects	Percentage
Insulin sensitivity (I.S)	I.S before treatment	16	53.33%
	New cases of I.S	4	13.33%
	Total No. after treatment	13	43.33%
Insulin resistance (I.R)	I.R before treatment	14	46.67%
	New cases of I.R	7	23.33%
	Total No. after treatment	14	46.67%
DM	Before treatment	0	0%
	New cases of DM	3	10%
	Total No. after treatment	3	10%
	U	384.00	
	P	> 0.05	
	Sig.	NS	

Table (3): Comparison between group C at beginning of the study and after 6 months of treatment as regard HCV, insulin sensitivity and resistance

HOMA-IR	**Before treatment**	**After treatment**		**X^2**	***p***	**Sig.**
	HCV +ve	**HCV -ve**	**HCV +ve**			
IR	14(46.67%)	1 (3.33%)	16(53.33%)	8.557	<0.01	**HS**
IS	16(53.33%)	11(36.67%)	2 (6.67%)			
Total	**30 (100%)**	**12 (40%)**	**18 (60%)**			

4. Discussion:

The World Health Organization (WHO) estimates that 170 million individuals worldwide are infected with hepatitis C virus (HCV), however the prevalence of HCV infection varies throughout the world. Egypt has the highest number of reported infections, with 22% seropositivity, largely attributed to the use of contaminated parenteral antischistosomal therapy [6]

Up to one third of patients with chronic hepatitis C virus (HCV) develop type 2 diabetes mellitus (DM). This prevalence is much higher than that observed in the general population and in patients with other chronic liver diseases such as

hepatitis B virus, alcoholic liver disease, and primary biliary cirrhosis. Further, HCV seropositivity in patients with DM appears to be higher than in the general population. Post liver transplantation DM also appears to be higher among patients with HCV [7].

In this study, the prevalence of insulin resistance and DM in chronic hepatitis C patients, was plotted against a control group of non diabetic patients without chronic hepatitis C and showed that the prevalence of insulin resistance is significantly higher in HCV patients and it is improved with successful antiviral therapy**.**

HOMA-IR was significantly correlated with BMI and waist circumference, and a highly significant correlation was found with AST, ALT, HCV- RNA level, and liver biopsy indices (inflammatory , steatosis , and fibrosis index.). Several studies have demonstrated insulin resistance in patients with HCV-related chronic hepatitis, using a homeostasis model assessment (HOMA) [4,7]**.** It was postulated that HCV infection promotes IR mainly through increased TNF-α production, which inhibits insulin receptor and IRS-1 (insulin receptor substrate) tyrosine phosphorylation [8]. Activation of the tumour necrosis factor (TNF) system has a pivotal role in the inflammatory process of chronic hepatitis C, and TNF- levels correlate with the degree of inflammation. TNF is known to cause insulin resistance, with similar defects in the insulin signalling pathway to those described in HCV infection [9].

Diabetic HCV patients have significantly higher levels of soluble TNF receptors, compared to non-diabetic HCV patients and controls. TNF may be the link between HCV infection and diabetes, suggesting an additional mechanism of diabetes with important implications for prognosis and therapy [10].

A recent study stated that hepatic insulin signaling is impaired in nonalcoholic steatohepatitis (NASH) and HCV patients, and down-regulation of insulin-sensitive targets is associated with increased apoptosis and fibrogenesis in both conditions. This might be a target for HCV-induced insulin resistance [11].

However the reasons why chronic HCV infection would induce type 2 diabetes could be manifold. Several experimental studies have suggested a direct role of the virus in promoting DM risk. Within HCV core-transgenic mice, hepatocyte-associated degradation of the HCV core protein leads to negative interaction with insulin signaling by reducing insulin receptor substrate-1 (IRS-1) phosphorylation [12], and by promoting IRS-1 and IRS-2 degradation [13] .The virus has also been localized in 39% of pancreatic islets in HCV-infected humans and occurs in approximately 54% of all cells within affected islets. Although there is no evidence of increased apoptosis, these HCV +ve islet cells exhibit morphologic changes as well as derangement in glucose-stimulated insulin release (β-cell dysregulation) [14]**.** The direct effect of HCV on pancreatic β-cells is controversial; some authors observed virus-like particles in pancreatic β-cells from HCV-infected patients and found a reduction in glucose-stimulated insulin release *in vitro* [14]**.** On the other hand, others detected a compensatory increase of pancreatic islet mass without infiltration of inflammatory cells in HCV core transgenic mice [15]**.** Clinically, many researchers have reported that insulin secretion is upregulated in HCV-infected patients to compensate for IR [3, 16] which is in good agreement with our findings. Therefore, we consider that insulin hypersecretion is a compensatory reaction caused by IR.

Interestingly, we noticed a significant increase in insulin resistance in HCV patients who did not receive treatment (11 new cases of insulin resistance after 6 months) or did not respond to treatment (16 out of 18 patients showed insulin resistance). On the other hand , 11 out of the 12 patients who turned HCV negative exhibited insulin sensitivity. This is in accordance with other studies stated that therapy with pegylated interferon and ribavirin is associated with a decrease in body weight and insulin resistance. Among patients with insulin resistance before treatment, resolution of HCV infection results in sustained improvements in the HOMA-IR index, suggesting that HCV could have a direct role in the pathogenesis of insulin resistance [17]. In a study analyzed improvements in insulin resistance after 24 weeks of pegylated interferon and ribavirin therapy, they demonstrated that clearance of virus with antiviral therapy was associated with the most pronounced reduction, and partial responses of virus were associated with intermediate reductions, supports a direct causal relationship between HCV and insulin resistance [18]**.**

5. Conclusion

Chronic hepatitis C virus infection is a risk factor for development of insulin resistance and DM, particularly in patients with visceral obesity. Viral eradication is associated with improved insulin sensitivity. We recommend close and long term monitoring of non-responder chronic HCV for potential increased risk of developing DM.

Corresponding author:
Amal Shawky Bakir MD
Faculty of Medicine , Ain Shams University.
Cairo, Egypt.
E.mail: amalshawky_mb@hotmail.com

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