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Peripheral Insulin Resistance During Treatment of Chronic Hepatitis C With Peguilated Interferon Plus Ribavirin

Vivianne Mello,[1]* Thomaz Cruz,[1,2,4] Geila Nuñez,[2] Maria Tereza Simões,[2] Fabrizio Ney-Oliveira,[2] Hélio Braga,[1,2] CésarAraújo,[3] Simone Cunha,[1] Maria Isabel Schinoni,[1] Marla Cruz,[4] and Raymundo Parana[1,2]

[1]*Post-Graduation Course in Medicine and Health, Federal University of Bahia, Bahia, Brazil*
[2]*Medical School, Federal University of Bahia, Bahia, Brazil*
[3]*Image Memorial Radiology Service, Salvador, Bahia, Brazil*
[4]*LEME Laboratory, Salvador, Bahia, Brazil*

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. The objective of this study was to investigate the relationship between the use of peguilated interferon and the development of insulin resistance (IR) in these patients. Methods: HOMA index was evaluated in 30 HCV-infected patients just before and during the first 6 months of treatment with peguilated interferon plus ribavirin. Anthropometrical parameters and glucose/cholesterol profile were also monitored. Results: No changes in HOMA after 6 months of treatment were observed. Glucose levels decreased but not significantly ($P = 0.059$). Patients with higher HOMA index after 6 months of treatment also presented higher aminotransferase levels ($P = 0.03$), higher fat index on computed tomography ($P = 0.011$), longer time of exposure to the virus ($P = 0.021$), and a positive smoking history when compared to non-insulin resistant patients ($P = 0.045$). There was no influence of fibrosis stage on liver biopsy in the insulin-resistance development. Conclusions: No changes in the IR were observed after 6 months of treatment. Insulin resistance is related to the abdominal fat and anthropometrical parameters rather than to the antiviral treatment. ***J. Med. Virol. 78:1406–1410, 2006.***

KEY WORDS: interferon; peripheral insulin resistance; virus c hepatitis; diabetes mellitus

INTRODUCTION

Association between hepatitis C virus (HCV) and type-2 diabetes mellitus (DM) has been suggested. Many studies have shown greater prevalence of type-2 DM in patients with chronic hepatitis C at levels significantly higher than in normal population [Knobler et al., 1993; Allison et al., 1994].

The pathogenesis of diabetes in patients with HCV is not well understood, but an important role of insulin resistance (IR) has been proposed as the link between these two pathologies. Higher prevalence of DM in HCV patients has been described in cirrhotic and non-cirrhotic patients [Hieronimus et al., 1997].

Cirrhotic patients have elevated post-prandial glucose and higher levels of circulating insulin [Marchesini et al., 1981; Who, 1985; Petrides et al., 1994.]. They also have a diminished response to exogenous insulin, when compared to normal controls [Petrides and Defronzo, 1989]. The higher prevalence of DM in HCV patients is not exclusively related to cirrhosis. Non-cirrhotic patients with chronic HCV have increased prevalence of type-2 DM compared to non-cirrhotic patients with chronic hepatitis B virus [Akbar et al., 2002].

A comparison between cirrhotic patients with hepatitis B virus and cirrhotic patients with HCV infection showed a higher prevalence of type-2 DM in patients with HCV infection (9.4% vs. 23.6%, $P = 0.0002$). The occurrence of diabetes in these patients was associated with the Child-Pugh score and age. Insulin concentrations were elevated

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*Correspondence to: Vivianne Mello, R. Almirante Barroso, no. 248, ap 27 BP, Cd. Bahia Bella, Ed. Bela Praia, Rio Vermelho, Salvador, Bahia, Brasil, CEP 41950-350.
E-mail: viviannemello@uol.com.br

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in 30 patients with cirrhosis associated with HCV infection and DM, suggesting IR as a possible cause for diabetes [Caronia et al., 1999].

The use of interferon has already been described as a cause for glucose metabolism disturbs during treatment of chronic hepatitis C, not only making the therapeutic for a pre-existing diabetes more difficult, but also precipitating the appearance of the DM [Fabris et al., 1992; Guerci et al., 1994; Durazzo et al., 1997]. On the other hand, a study evaluated the effects of interferon-α on the glucose metabolism in patients with chronic hepatitis B and C infections, and the steady-state plasma glucose of the insulin suppression test decreased significantly in responders 3 months after completion of the interferon-α treatment [Tai et al., 2004].

The objective of this study is to verify if interferon itself induces IR and to investigate the associated clinical, biochemical, epidemiological, and viral factors.

PATIENTS AND METHODS

Population

Thirty patients (mean age ± SD = 45.47 ± 8.5 years) with chronic hepatitis C consecutively followed at the Professor Edgard Santos Hospital in Brazil (March 2003–2004) were enrolled. The study protocol is in agreement with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Institution's Human Research Committee.

Inclusion Criteria

Age between 18 and 65 years, no use of antiviral medications for the last 6 months, positive HCV-RNA, histological evidence of necro-inflammation and fibrosis evaluated by METAVIR score [Bedossa and Poynart, 1996], with necro-inflammatory index ≥2, and/or fibrosis index ≥2, and acceptance in participating in this study by signing the consent term.

Exclusion Criteria

Patients with DM, contra-indications for hepatic biopsy, and use of medications that could induce the development of IR or the non-concordance in signing the consent term.

Evaluation of the Study Subjects

All patients were evaluated before starting the use of peguilated interferon-α 2a or 2b plus ribavirin, and after 3 and 6 months from the beginning. At the first clinical evaluation, information about gender, race, blood transfusion, time of infection, age, symptoms, previous use of antiviral medications, and if the patient had ever used interferon, time of use and kind of response, if non-responder (a positive HCV-RNA at end of the treatment) or relapsing (a negative HCV-RNA at end of the treatment), fibrosis index in liver biopsy, personal and familiar history of arterial hypertension, obesity, and dislypidemia. A physical examination was performed, with special attention for blood pressure, body mass index (BMI), and waist circumference. BMI was calculated by the Quetelet index: weight in kilograms/height in meters^2 [Willet et al., 1999]. Waist circumference was measured with a non-elastic measuring tape, in centimeters [Jebb and Disord, 1993]. Other physical examinations were performed 3 and 6 months after medications started being used.

TABLE I. Demographic Data

Variable	Patients(n = 30)
Age (years) [a]	45.47 ± 8.5
Gender n (%)	
Male	25 (83.33%)
Female	5 (16.67%)
Genotype, n (%)	
Genotype 1	25 (83.33%)
Genotype 3	(16.67%)
Exposition time, n (%)	
10–19 years	7 (23.33%)
More than 20 years	11 (36.67%)
Unknown	12 (40%)
Previous use of medications, n (%)	
Never used	5 (16.67%)
Previous use of interferon	25 (83.33%)

[a]Data are presented as mean ± SD.

Insulin Resistance (IR)

Insulin resistance was calculated through the HOMA method as follows: HOMA = fasting serum insulin (μU/ml) × serum glucose (mMol/L)/22.5. Patients were categorized as insulin resistant if HOMA was greater than 2, as defined by a healthy control group [Matthews et al., 1985].

Laboratory Analysis

Sera were stored at −20°C until analyses. A qualitative HCV-RNA (reverse transcription-polymerase chain

TABLE II. Demographic Data

Variable	Patients(n = 30)
Fibrosis index, n(%)	
F1	3 (10%)
F2	8 (26.67%)
F3	11 (36.67%)
F4	8 (26.67%)
Hepatic biopsy, n(%)	
Steatosis	11 (36.67%)
Iron overload	2 (6.67%)
Both	2 (6.67%)
Medical history, n(%)	
Arterial hypertension	2 (6.67%)
Obesity	2 (6.67%)
Dyslipidemia	1 (3.33%)
Smoking	8 (26.67%)
Alcohol use	6 (20%)
Familiar history, n(%)	
Diabetes	14 (46.67%)
Hypertension	21 (70%)
Obesity	6 (20%)
Dyslipidemia	9 (30%)
Fat index on CT (cm^2)[a]	
Total abdominal fat	311.33 ± 127.02
Visceral abdominal fat	103.94 ± 49.79
Subcutaneous abdominal fat	206.53 ± 95.13

[a]Data are presented as mean ± SD.

TABLE III. BMI and Waist Circumference Means During Treatment

	Baseline	Week 12	Week 24	*P*1	*P*2
BMI (kg/m^2)	25.83 ± 3.93	25.15 ± 4	24.59 ± 3.91	<0.001	0.005
Waist circumference	87.52 ± 10.7	87.52 ± 10.67	87.09 ± 10.18	0.077	0.011

Data are presented as mean ± SD. Table III. *P*1, significance on comparison between baseline and values of week 12. *P*2, significance on comparison between baseline and values of week 24.

reaction) with genotype was performed for all patients [Silva et al., 2000]. After a 12 hr fast, venous blood samples were obtained to measure levels of the following: fasting insulin, insulin 2 hr after oral intake of 75 g of glucose (Human Insulin RIA kit, LINCO Research, Inc.; St. Charles, MO); fasting glucose and after oral intake of 75 g of oral glucose; alanine aminotransferase; aspartate aminotransferase; cholesterol; uric acid, triglycerides; transferrin saturation (all from Vitros Chemistry, Ortho Clinical Diagnostics, Johnson&Johnson; NY); ferritin (Ferritina, Bayer; SP, Brazil) and glicosilated hemoglobin (Hemoglobin A1DC Bio-Rad, Bio-Rad; Hercules, CA).

Image Methods Evaluation

In order to quantify intra-abdominal obesity, a simple abdominal CT was performed at level between the fourth and fifth lumbar vertebras. Visceral and subcutaneous fat areas were delimitated with a linear cursor and then quantified. Fat areas were described in centimeter^2 [Enzi and Gasparo, 1986].

Statistical Analysis

Continuous variables are categorized as mean ± stanstandard deviation and categorical variables as frequencies and percentages. For comparison between independent variables, the Mann–Whitney test was used. For dependent variables, the Wilcoxon test was performed. Non-continuous variables were compared through the Chi-Squared test. All analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL). A significance level of 5% was adopted.

RESULTS

Patient's demographic data are shown in Tables I and II.

After 3 months of treatment, 18 patients (60%) were still positive for HCV-RNA. Seven patients stopped use of medications after 18 weeks of treatment because of non-viral response. Although presenting no negative HCV-RNA, 11 patients continued using medications until week 24. For this reason, a drop out of 7 patients was observed between weeks 12 and 24 of treatment. All patients with positive HCV-RNA on week 12 remained positive on week 24.

We compared blood pressure, BMI, and waist circumference values obtained before therapy used for those at the 3rd and 6th months. A decrease on BMI at the third and 6th months was observed ($P < 0.0001$ and $P = 0.005$ respectively). Waist circumference also decreased after 6 months of treatment ($P = 0.0011$) (Table III).

Biochemical Evaluation

A significant decrease on the transaminase levels during treatment was verified. Serum glucose levels also decreased significantly after 3 months. There was a slight elevation on the glucose levels between weeks 12 and 24, but with no statistical significance. Significant changes in HOMA results, fasting insulin, glucose after oral glucose intake or insulin after oral glucose intake levels were observed after 3 or 6 months of treatment. Ferritin levels also increased during treatment. These values are shown in Table IV.

Insulin Resistance (IR)

Baseline. Using HOMA ≥2 for defining IR on baseline, we found 16 (53%) patients with IR. These patients presented higher BMI ($P = 0.034$); waist circumference ($P = 0.031$); fasting insulin levels ($P < 0.001$); non-fasting insulin levels ($P = 0.008$); glicosilated hemoglobin ($P = 0.048$); total and subcutaneous abdominal fat indexes on CT ($P = 0.018$ and $P = 0.008$, respectively) when compared to patients without IR. No other parameter differed significantly between the two groups (see Table V).

Week 12. After 12 weeks of treatment, 15 (50%) patients out of the 30 patients evaluated presented IR.

TABLE IV. Biochemical Evaluation

	Baseline	Week 12	Week 24	*P*1	*P*2
Fasting glucose (mg/dl)	92.57 ± 9.74	85.5 ± 10.11	87.96 ± 12.97	<0.001	0.06
Non-fasting glucose (mg/dl)	110.1 ± 27.78	109.23 ± 32.36	111.57 ± 36.54	0.93	0.65
HOMA	2.42 ± 1.56	2.14 ± 1.26	2.03 ± 1.51	0.98	0.48
Fasting Insulin (μU/L)	10.53 ± 6.61	10.01 ± 5.78	9.27 ± 7.39	0.69	0.26
Non-fasting insulin (μU/L)	61.66 ± 34.64	60.10 ± 40.3	72.47 ± 90.50	0.35	0.78
Ferritin (ng/ml)	452.13 ± 341	787.37 ± 689.14	639.46 ± 412.87	0.0003	0.003

Data are presented as mean ± SD. *P*1, significance on comparison between baseline and values of week 12. *P*2, significance on comparison between baseline and values of week 24.

TABLE V. Insulin Resistance on Baseline

Variable	NIR	IR	*P*
BMI (kg/m^2)	24.19 ± 2.18	27.27 ± 4.58	0.034
Waist (cm)	82.78 ± 8.67	91.68 ± 10.74	0.031
Fasting glucose (mg/dl)	91.07 ± 6.69	93.88 ± 11.87	0.59
Fasting insulin (μU/ml)	6.41 ± 1.51	14.13 ± 7.27	<0.001
Non-fasting insulin (μU/ml)	43.26 ± 20.82	77.76 ± 36.72	<0.001
Glicosilated hemoglobin (%)	5.78 ± 0.66	6.2 ± 0.56	0.048
Total abdominal fat on CT (cm^2)	249.24 ± 77.71	359.1 ± 139.15	0.018
Visceral abdominal fat on CT (cm^2)	89.19 ± 45.33	115.28 ± 51.81	0.18
Subcutaneous fat on CT (cm^2)	158.05 ± 47.65	246.82 ± 106.89	0.008

Data are presented as mean ± SD. IR, insulin resistant; NIR, non-insulin resistant.

Insulin-resistant patients on week 12 presented higher ALT levels on baseline ($P = 0.0023$) and on week 12 ($P = 0.016$) than non-insulin resistant patients. They also presented higher fasting insulin levels on week 12 ($P < 0.001$), post-glucose insulin levels on week 12 ($P = 0.010$), and ferritin on week 12 ($P = 0.026$) than non-insulin resistant patients. Patients with IR after 12 weeks of treatment also presented more familiar hypertension history than non-insulin resistant patients ($P = 0.014$). No further differences between these two groups were found.

Week 24. At this point, the IR in 23 patients has already been evaluated. After 24 weeks of peguilated interferon use, 7 of the 23 remaining patients still presented IR. Patients with IR in the 6th month presented an almost significant larger waist circumference on baseline than non-insulin resistant patients ($P = 0.053$). At the 6th month, insulin-resistant patients also presented higher levels of fasting insulin ($P = 0.001$), non-fasting insulin ($P = 0.005$), waist circumference ($P = 0.035$), serum ALT ($P = 0.003$), serum AST ($P = 0.030$), fasting glucose ($P = 0.035$), non-fasting serum glucose ($P = 0.005$), visceral fat index on CT ($P = 0.002$) and total fat index on CT ($P = 0.035$) than non-insulin resistant patients. Insulin-resistant patients also presented earlier exposition to HCV, and more smoking history than non-insulin resistant patients. No statistical difference was observed between both groups in relation to the fibrosis level. No other difference was verified in these two groups. (Table VI)

HOMA. The Mann–Whitney test was performed for the evaluation of the HOMA index. Patients with more than 20 years since the probable infection event presented higher HOMA index at the 6th month of treatment ($P = 0.032$). Patients who had already used interferon also presented a significantly higher HOMA index than patients who had never used antiviral drugs ($P = 0.036$). See Table VII.

DISCUSSION

Insulin resistance seems to be the link between HCV and DM. On the other hand, interferon effects on the glucose metabolism are not clear. It is not known if interferon may worse or even cause IR, or as recently demonstrated, if patients who respond to treatment involve to a better glucose metabolism [Tai et al., 2003].

The objective of this study was to demonstrate the relationship between the use of peguilated interferon and IR in chronic HCV infection.

No change in the HOMA levels during 6 months of treatment was verified, and the glucose levels decreased significantly after 3 months of interferon use. Six months of peguilated interferon plus ribavirin treatment did not cause IR. In contrast, the glucose metabolism improved during this treatment period.

BMI and waist circumference decreased during treatment. This may be due to the loss of weigh as an adverse effect of the medication use. The loss of weight may be related to the decrease on the insulin levels, since abdominal fat is related with diminished insulin-mediated glucose release [Abbasi et al., 2002].

According to other authors, patients who presented IR on baseline had more pronounced waist circumference and higher BMI [Duong et al., 2001].

TABLE VI. Insulin Resistance After 6 Months of Treatment

Variable	RI (Mean ± SD)	NRI (Mean ± SD)	*P*
Waist circumference at baseline (cm)	95.5 ± 8.76	84.53 ± 10.1	0.053
Waist circumference at week 24 (cm)	94 ± 9.17	83.09 ± 8.94	0.035
Fasting glucose at week 24 (mg/dl)	96.57 ± 12.9	89.56 ± 7.87	0.035
AST at week 24(UI/L)	109.57 ± 87.75	40.31 ± 22.63	0.03
ALT at week 24(UI/L)	129.86 ± 105.9	43.5 ± 25.96	0.003
Total abdominal fat on CT (cm^2)	430.23 ± 139.4	259.75 ± 108.74	0.011
Visceral abdominal fat on CT (cm^2)	85.02 ± 44.59	158.14 ± 37.38	0.002

IR, insulin resistant; NIR, non-insulin resistant.

TABLE VII. HOMA X Exposition Time and Interferon Use

Variable	HOMA on week 24 (Mean ± SD)	*P*
Previous interferon use		
Yes	2.18 ± 1.57	0.036
No	1.03 ± 0.24	
Exposition time		
10–19 years	12.26 ± 19.78	0.032
>20 years	33.95 ± 26.08	

The finding of a greater index of total and visceral fat and abdominal fat on CT in insulin-resistant patients supports literature reports, so the action of insulin is more determined by the visceral abdominal fat, and less by the subcutaneous fat in HCV carriers and non-carriers.

After 6 months of treatment, there was no change in the IR, but some peculiarities were observed in patients who presented IR after 6 months of peguilated interferon plus ribavirin treatment. These patients presented higher ALT levels. We do not know if the higher serum transaminase levels found is related to a poor viral response because we did not follow patients for evaluation of sustained viral response. These patients also presented increased visceral and total abdominal fat on tomography, indicating an effect of abdominal obesity on IR.

Patients with probable longer time of acquisition of virus C infection were at greater risk for developing IR during peguilated interferon plus Ribavirin treatment. Curiously, we found IR was more frequent among patients who had been treated with interferon plus ribavirin before. Thus, previous interferon treatment could induce IR by an immune-mediated way [Abbas et al., 2002].

There are still many obscure points concerning the relation of IR and interferon plus ribavirin treatment. More clear evidences are necessary for the understanding of this complex relation. Attention should be given to patients who seem to be at greater risk for IR, including those presenting no reduction of ALT during treatment, obese patients, those with greater inflammatory activity (METAVIR), and those with more than 20 years of exposition to HVC.

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