Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C

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Background/Aims: To investigate the effect of sustained virological response (SVR) on impaired fasting glucose (IFG) and/or type 2 diabetes (T2DM); to assess the influence of glucose abnormalities on the SVR rate.

Methods: 1059 patients with chronic HCV; normal glucose (< 100 mg/dl) in 734, IFG (between 100 and 125 mg/dl) in 218, and T2DM (≥ 126 mg/dl) in 107 cases, were treated with interferon plus ribavirin over 24 or 48 weeks, depending on viral genotype.

Results: The SVR rate was lower in patients with IFG and/or T2DM than in patients with normal glucose concentrations [143/325 (44%) vs. 432/734 (58.8%); P = 0.002]. In the follow-up, abnormal glucose concentrations were observed in 74 of 304 (24.3%) non-responders and in 49 of 430 (11.4%) sustained responders (log-rank: 13.8; P = 0.00002). Reverse stepwise logistic regression analysis identified the independent variables predictive of IFG or T2DM development as: sustained response (OR: 0.44; 95%CI = 0.20–0.97; P = 0.004) and fibrosis stage (OR: 1.46; 95%CI = 1.06–2.01; P = 0.02). Family history of DM, steatosis, gender, HCV viral load, genotype, triglycerides, cholesterol and BMI did not enter the multivariate analysis equation.

Conclusions: SVR reduces the risk of IFG and/or T2DM development in patients with chronic hepatitis C while altered glucose metabolism impairs sustained response to viral treatment.

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1. Introduction

The development of type 2 diabetes mellitus depends on environmental, genetic and diet-related factors. Data supporting a link between hepatitis C infection, insulin resistance and type 2 diabetes mellitus [1] include: (1) insulin resistance is higher in chronic hepatitis C than in other chronic liver diseases, despite the same fibrosis stage, body mass index, age and family history of diabetes [2]; (2) in transgenic mice, the expression of HCV core protein is associated with insulin resistance, following a fat-enriched diet, and with type 2 diabetes development [3]; (3) clearance of the virus following successful treatment is related to a fall in the insulin resistance index measured by the homeostasis model of assessment (HOMA) [4]; (4) cross-sectional studies have found higher prevalence of diabetes mellitus in patients with chronic hepatitis C than in healthy subjects, or in those suffering from other chronic liver diseases [5]; (5) case-cohort studies have demonstrated a higher incidence of type 2 diabetes mellitus during follow-up in patients with hepatitis C, as compared to non-infected subjects [6]. The mechanisms by which hepatitis C impairs insulin sensitivity and promotes diabetes include: increased tumour necrosis factor (TNF-α) production [7] and over-expression of suppressor of cytokines signal 3 (SOCS-3) [8] which have been shown to interfere with the intracellular signalling pathway of insulin.

The main aim of the current study was to assess the effect of sustained virological response, together with host and viral factors, on the incidence of impaired fasting glucose and/or type 2 diabetes mellitus in patients with chronic hepatitis C being treated with combined interferon plus ribavirin. Further, we sought to assess the impact of baseline glucose status on sustained virological response rate.

2. Patients and methods

2.1. Patient population

Patients from 11 Spanish hospitals were recruited and treated according to routine clinical practice. Patients (n = 1059; male = 649 (61.2%) and females = 410 (38.8%); mean age = 48 ± 11 years, range = 20–75) with biopsy-proven chronic hepatitis C and HCV RNA positive were recruited. They were receiving treatment with combination therapy of standard interferon plus ribavirin (n = 183), or peginterferon alfa-2a plus ribavirin (n = 501), or peginterferon alfa-2b plus ribavirin (n = 368). Viral genotype distributions were genotype 1 (n = 774), genotype 2 (n = 44), genotype 3 (n = 156), genotype 4 (n = 47), genotype 5 (n = 3). Genotype analysis was not available in 35 patients. Family history of type 2 diabetes mellitus (first degree relatives diagnosed) was present in 304 patients (28.7%). Risk factors for hepatitis C infection were history of blood transfusion in 257 (24.3%) patients, drug abuse in 171 (16.1%), tattooing in 31 (2.9%), 133 (12.6%) were health-care workers. The aetiology was unknown in 467 (44.1%) of cases. The average duration of the disease was 20 ± 9 years. Fully informed written consent to participation was obtained prior to entry into the study. The Hospitals’ Ethics Committee approved the protocol.

2.2. Laboratory investigations

An overnight (12 h) fasting blood sample was taken for routine analyses. These included transaminase activities, platelet counts, and cholesterol and triglyceride concentrations. All patients had positive anti-HCV as measured using EIA3 (Abbott Laboratories, Chicago, IL), increased ALT, and positive HCV RNA in serum. HBsAg, anti-HBc, anti-HIV were measured using commercially available kits (Abbott Laboratories, Chicago, IL). All patients were negative for HBsAg and anti-HIV.

Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg) / height (in m²).

2.3. Liver histology

Percutaneous liver biopsy was performed under ultrasound control. We assessed grading and staging separately. The stage was defined according to the Scheuer fibrosis score in which F0 = absence, F1 = enlarged portal tracts, F2 = peri-portal or portal-portal septa, F3 = fibrosis with architectural distortion, and F4 = cirrhosis. Necro-inflammatory activity was determined by combining scores for portal inflammation (scale 0–4) and lobular necrosis (scale 0–4). Steatosis was present in 592 cases and was quantified as the percentage of hepatocytes that contained fat droplets; classification being presence (>2% of hepatocytes with fat droplets) or absence.

2.4. Virological determinations

HCV genotyping was performed with INNO-LIPA HCV II kits (Innoogenetics, Zwijnaarden, Belgium) according to the manufacturer’s instructions. An Ampli-cor-HCV-Monitor 2.0 (Perkin-Elmer, Norwalk, CT) was used to quantify the HCV RNA levels in serum. Serum samples were diluted for measurement when values were beyond the linear range of the method. The level of detection was 50 IU/mL, and all patients were HCV RNA positive.

2.5. Treatment outcomes

Patients were treated according to viral genotype for 24 weeks (genotype 2, 3) or 48 weeks (genotype 1, 4). Patients previously diagnosed as having type 2 diabetes mellitus, taking oral hypoglycaemic drugs or insulin, or having a fasting glucose concentration ≥100 mg/dl were not included in the follow-up analysis. Time zero was start of treatment and the end point was the development of abnormal fasting glucose concentration and/or type 2 diabetes mellitus by the end of follow-up. Patients were monitored every six months over the 27 ± 17 months (range: from 9.3 to 67 months) of follow-up. At the end of follow-up, patients were asked about their use of hypoglycaemic drugs and recent diagnosis of T2DM. Fasting glucose was measured at each clinical consultation visit. Patients were classified as having type 2 diabetes mellitus (glucose ≥126 mg/dl), impaired fasting glucose (fasting glucose level of 100–125 mg/dl) or normal glucose metabolism (fasting glucose <100 mg/dl).

2.6. Statistical analyses

The χ² test was used to assess differences between qualitative variables. The Levene test was used in the evaluation of differences in variance. ANOVA and the Student t-test were used to compare the means of quantitative values of glucose and to analyse factors predicting sustained response. Reverse stepwise logistic regression was used for multivariate analysis. Spearman or Pearson coefficients were used to compare quantitative variables. Kaplan–Meier analysis was used for univariate analysis and Cox-regression for multivariate analysis of variables associated with the appearance of impaired fasting glucose in the course of the follow-up. The SPSS package (SPSS 11.5 for Windows, SPSS, Chicago, IL) was used for all the statistical analyses, and in generating the figures.
3. Results

3.1. Factors associated with sustained virological response

Patients with SVR were younger (46.2 ± 10.4 vs. 50.4 ± 10.4 years; \( P < 0.001 \)), had lower glucose concentrations (5.4 ± 3.1 mmol/L vs. 7.5 ± 1.58 mmol/L; \( P < 0.001 \)), lower baseline HCV RNA load (5.74 ± 0.6 \( \log_{10} \) vs. 5.88 ± 0.49\( \log_{10} \); \( P < 0.05 \)), lower triglyceride concentrations (1.05 ± 0.66 mmol/L vs. 1.14 ± 0.79 mmol/L; \( P < 0.05 \)), and higher total cholesterol concentrations (4.48 ± 1.0 mmol/L vs. 4.31 ± 0.96 mmol/L, \( P < 0.005 \)). There were no significant associations with gender, body mass index, and levels of ALT, AST or platelets. SVR was achieved in 47.6% (367 of 770) patients with genotype 1 vs. 75.7% (150 of 198) patients with genotype 2,3 (\( P < 0.0001 \)). The SVR rate was 56.8% (412 of 725) patients with mild fibrosis (F0–F2) and 40.1% (89 of 223) patients with advanced fibrosis (F3–F4); \( P < 0.001 \). In patients without steatosis the SVR rate was 61.2% but in patients with steatosis SVR was achieved in 102 of 215 (46.6%); \( P < 0.001 \). With respect to glucose concentrations, SVR was observed in 432 of 734 patients (58.8%) with normal glucose metabolism and in 143 of 325 patients (44%) with abnormal glucose values, \( P < 0.001 \). However, no significant differences were observed in the sustained response rate in patients with abnormal glucose (\( \geq 100 \) mg/dl): SVR was seen in 62 of 135 (45.9%) patients with fasting glucose between 100 and 110 mg/dl; in 35 of 83 (42.2%) patients with fasting glucose between 110 and 125 mg/dl, and in 46 of 107 (43%) patients with type 2 diabetes mellitus (\( \chi^20.36, P = 0.38 \)).

Using reverse stepwise logistic multivariate regression analysis, the independent variables related to SVR were genotype 2, 3 (OR: 4.64; 95%CI = 2.54–8.50; \( P < 0.0001 \), fasting plasma glucose (\( > 100 \) mg/dl) (OR: 0.56; 95%CI = 0.34–0.93; \( P < 0.02 \), steatosis (OR: 0.57; 95%CI = 0.35–0.92; \( P < 0.02 \), baseline HCV RNA load (OR: 0.42; 95%CI = 0.27–0.65; \( P < 0.0001 \)) and age (OR: 0.96; 95%CI = 0.93–0.98; \( P < 0.0007 \)) (Table 1).

3.2. Prevalence of impaired fasting glucose, type 2 diabetes and related factors in chronic hepatitis C

Prior to the current treatment, 107 patients (10.1%) had been diagnosed as having type 2 diabetes mellitus, and impaired fasting glucose was observed in 83 patients (7.8%) with baseline glucose concentrations of between 110 and 125 mg/dl, and 135 patients (12.7%) with baseline glucose between 100 and 110 mg/dl). Lastly, 734 patients (69.3%) had normal glucose concentrations (<100 mg/dl). Impaired fasting glucose and/or diabetes mellitus prior to treatment were related to advanced age (52.9 ± 9.7 vs. 45.7 ± 10 years; \( P < 0.001 \)), higher body mass index (27.7 ± 4.1 vs. 25.5 ± 4.0 kg/m\(^2\); \( P < 0.001 \)), higher triglyceride concentrations (1.22 ± 0.90 mmol/L vs. 1.03 ± 0.62 mmol/L; \( P < 0.001 \)), lower platelet count (184 ± 70 vs. 207 ± 109 × 1000 platelets/mL; \( P < 0.001 \)), and higher AST activity (76.9 ± 60.5 vs. 65.1 ± 46.9 IU/mL; \( P < 0.001 \)). Patients with a family history of type 2 diabetes had a higher frequency (\( P < 0.001 \)) of abnormal glucose (84 of 194 patients, 43.3%) than those without (142 of 483 patients, 29.4%). Ninety-one of two hundred and fifteen patients (42.1%) with steatosis on liver biopsy had abnormal glucose metabolism compared to 93 of 377 (24.6%) patients without steatosis. Lastly, abnormal glucose values were observed more often in patients with advanced fibrosis (91 of 223 patients, 40.8%) than in patients with mild fibrosis (205 of 728, 28.2%; \( P < 0.001 \)). No statistically significant relationships were observed between abnormal glucose values and gender, cholesterol concentrations, HCV viral load, ALT activity, and genotype (Table 2).

In reverse stepwise multivariate logistic regression analysis, the variables associated with impaired fasting glucose and/or type 2 diabetes mellitus were age (OR: 1.08; 95%CI = 1.05–1.10; \( P < 0.001 \)), body mass index (OR: 1.12; 95%CI = 1.05–1.19; \( P < 0.001 \)) and steatosis (OR: 1.77; 95%CI = 1.15–2.71; \( P < 0.05 \)).

3.3. Factors associated with the development of type 2 diabetes mellitus and/or impaired fasting glucose following antiviral treatment in patients without abnormal baseline glucose

In the cohort of patients with normal glucose metabolism (baseline glucose <100 mg/dl, \( n = 734 \)) 124 cases (16.9%) developed glucose abnormalities after an average follow-up of 27 ± 17 months; 7 developing type 2 diabetes mellitus (all of them non-responders), 30 having impaired fasting glucose between 110 and 125 mg/dl (22 non-responders and 8 sustained responders) and 87 having impaired fasting glucose between 100 and 110 mg/dl (45 non-responders and 42 sustained responders) (\( \chi^229.7, P < 0.0001 \)). In univariate analysis, factors associated with the development of IFG and/or T2DM were body mass index (HR: 1.09; 95%CI = 1.03–1.15; \( P = 0.002 \)), steatosis (HR: 1.79; 95%CI = 0.96–3.35; \( P = 0.06 \)), fibrosis (HR: 1.14; 95%CI = 0.96–1.37; \( P = 0.08 \), and sustained response (HR: 0.45; 95%CI = 0.29–0.69; \( P = 0.0003 \)) (Fig. 1). No statistically significant associa-
tions were observed with family history of diabetes, genotype, risk factor for hepatitis C, duration of infection, HCV viral load, triglycerides, cholesterol, gender, or AST levels (Table 3). In the Cox reverse stepwise multivariate regression analysis the independent variables associated with altered glucose metabolism in the follow-up period were SVR (OR: 0.44; 95%CI = 0.20–0.97; \( P = 0.04 \)) and fibrosis stage (OR: 1.46; 95%CI = 1.06–2.01; \( P = 0.02 \)).

4. Discussion

Our results showed that the eradication of hepatitis C virus reduced by half the incidence of type 2 diabetes and/or impaired fasting glucose in the course of post-treatment follow-up. Further, sustained response and fibrosis stage were the variables that were independently predictive of the development of abnormal glucose concentrations. The significance was maintained even when analysed together with variables that are known to be strongly related to the risk of developing diabetes such as age, high body mass index, or diabetes in first-degree relatives [9]. These results are in accordance with the data reported by Simó et al. showing a reduction in the incidence of abnormal glucose values following treatment in a cohort of 234 cases [10] with chronic hepatitis C. Further, in a cohort of treatment-naïve chronic HCV patients recruited to receive treatment with standard interferon with or without ribavirin, the insulin sensitivity and β-cell function improved in sustained responders but remained unchanged in non-responders and relapers. Also, immunoblotting demonstrated a threefold increase in the expression of insulin receptor substrate-1 and insulin receptor substrate-2 relative to the baseline expression in patients who achieved SVR [11]. Hence, these findings need to be added to the well-established epidemiological [12] and molecular [3]
data demonstrating a link between hepatitis C and diabetes.

In previous studies, we had demonstrated that insulin resistance falls in sustained responders [4], but not in non-responders, following treatment and which supports the observation that the incidence of abnormal glucose values decreases after hepatitis C virus clearance. Although insulin resistance and diabetes occur more frequently in hepatitis C than in other chronic liver diseases regardless of the fibrosis stage [2], and more so than in healthy controls matched for age, gender, visceral obesity and body mass index [13], the mechanisms by which hepatitis C virus promotes insulin resistance are not well understood. Pazienza et al. reported a genotype-dependent mechanism for the development of insulin resistance in patients with hepatitis C. In genotype 1 the mammalian target of rapamycin (mTOR) and in genotype 3a the suppressor of cytokines signal 7 (SOCS-7) together with peroxisome proliferator-activated receptor γ (PPARs-γ) have been implicated in the development of insulin resistance in each of these genotypes [14]. Insulin resistance and diabetes have been associated with more rapid fibrosis progression [15]. Indeed, in the current study, fibrosis stage together with sustained response were found to be independently associated with a higher risk for impaired fasting glucose or diabetes development following treatment for the viral infection. Hyperinsulinaemia in liver cirrhosis could be due to diminished hepatic insulin removal by the dysfunctional liver. However, C-peptide and insulin are secreted in equimolar quantities and >50% of insulin is degraded in the first pass through the liver, whereas C-peptide is degraded in the kidneys [16]. Simultaneous measurements of C-peptide and insulin indicated that insulin resistance as well as insulin secretion contribute to glucose intolerance in patients with chronic hepatitis C [17]. Transgenic mice expressing core hepatitis C virus protein developed insulin resistance, while wild-type mice did not. In addition, a fat-rich diet induced type 2 diabetes in transgenic, but not in wild-type mice. Hepatitis C virus could induce the over-production of TNF-α, which is responsible for phosphorylation of serine residues of insulin-receptor substrates 1 and 2, and the enhancement of the production of cytokine suppressor signal 3 (SOCS3). Also, blocking TNF-α production by anti-TNF drugs such as infliximab prevents the development of insulin resistance in this model [3].

Achieving sustained response depends on several factors such as age, baseline viral load, genotype, fibrosis and baseline glucose concentrations. In patients with abnormal glucose values the rate of sustained response is 15% lower than in patients with fasting glucose below 100 mg/dl. The American Diabetes Association proposed changing the threshold defining impaired fasting glucose from 110 to 100 mg/dl [18]. However, this proposal is not universally accepted since patients with baseline glucose values between 100 and 110 mg/dl show no increased risk for cardiovascular disease [19], even in postmenopausal women with previous coronary artery disease [20]. Indeed, the European position on this issue is to recommend retaining the previous threshold of 110 mg/dl [21]. In the current study, we found a difference in the sustained response rate in patients with normal baseline glucose versus patients with impaired fasting glucose, but no difference was observed in patients with impaired fasting glucose once segregated according to fasting glucose bands of 100 to 110 mg/dl, 110 and 125 mg/dl and ≥126 mg/dl. This supports the usefulness of the current American Diabetes Association definition of impaired fasting glucose in predicting sustained response in patients with chronic hepatitis C treated with the interferon plus ribavirin combination.

In the current study, abnormal glucose values were detected more often in chronic hepatitis C, in older patients, those with steatosis, and those patients who were overweight. Interestingly, all three factors are related to insulin resistance. Indeed, insulin resistance was shown to increase in older patients, and to correlate
with steatosis severity and with body mass index [22]. This aspect could explain, at least in part, the elevated prevalence of impaired fasting glucose [23] and diabetes [24] in patients with chronic hepatitis C, compared to the general population. This provides further evidence of a link between hepatitis C and abnormal glucose metabolism and supports the concept of the virus being able to induce the development of diabetes in high-risk cases [9].

The criteria for the diagnosis of diabetes mellitus include: (a) random plasma glucose $\geq 11.1$ mmol/L; (b) fasting plasma glucose $\geq 7$ mmol/L; (c) 2 h post-load glucose (oral glucose tolerance test) $\geq 11.1$ mmol/L. Elevated fasting glucose results from raised hepatic glucose output and a defect in early insulin secretion, while peripheral insulin resistance is the most important characteristic of impaired glucose tolerance. The concordance between both tools has been reported as $<70\%$ and, while the impaired tolerance test seems to be more sensitive but less specific than impaired fasting glucose [25], both are similarly associated with an increased risk of overt diabetes [26]. The strengths of the current study are its multi-centred nature and the large numbers of patients included. However, these results need to be confirmed in further studies with a longer-term follow-up of patients at high risk of developing diabetes.

In conclusion, the antiviral treatment of hepatitis C infection, when successful in the eradication of the virus, induces a decrease in the incidence of diabetes and/or impaired fasting glucose. Approximately 5% of diabetic patients are infected with hepatitis C. However, in the near future a large number of patients with hepatitis C will be $>50$ years of age and they could develop diabetes if they remain uncured of the viral infection. Whether this could have any impact on the outbreak of diabetes in Western countries is yet to be determined. As has been reported previously with respect to insulin resistance, abnormal glucose values ($\geq 100$ mg/dl) have been shown in our study to be associated with a lower rate of SVR to treatment. The question of whether intervention using oral hypoglycaemic drugs, or diet and exercise, improves the response rate in this group of patients warrants further exploration.

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References