APORTACIONES ORIGINALES

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Hepatitis *C* virus infection

and type 2 diabetes mellitus in Mexican patients

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Resumen

Objetivo: probar que la diabetes mellitus tipo 2 (DM2) es frecuente en mexicanos con infección por virus de la hepatitis *C* (VHC).

Métodos: 125 adultos positivos a anticuerpos antiVHC (62.4 % mujeres), con pruebas confirmatorias para viremia mediante RT-PCR (63.2 % positivos al ARN del VHC).

Resultados: 22 pacientes presentaron DM2 (17.6 %, IC 95 % = 11.8-25.3); la diabetes fue más frecuente en pacientes con viremia detectable que en negativos (23.3 *adversus* 9.6 %, *p* = 0.04), y entre aquellos con enfermedad hepática avanzada que en compensados (28.9 *adversus* 11.3 %, *p* = 0.01). Catorce pacientes (17.7 %) recibieron interferón y seis (42.8 %) experimentaron respuesta virológica sostenida; ninguno de los últimos presentaron DM2; dos de los ocho (25 %) no respondedores tuvieron diabetes. Los pacientes con DM2 eran más añosos que aquellos sin diabetes (57.7 *adversus* 44.5 años, *p* < 0.001). Después del análisis multivariado ajustado por confusores relevantes, solo la edad se asoció significativamente con DM2.

Conclusiones: la DM2 fue altamente prevalente entre pacientes con infección por el VHC. La edad fue el factor determinante en la ocurrencia de diabetes en este grupo no pareado.

Palabras clave
diabetes mellitus tipo 2
hepatitis C
hígado

Summary

Objective: to measure the frequency of type 2 diabetes mellitus (T2DM) in patients with confirmed HCV infection.

Methods: we studied 125 adults reactive to anti-HCV antibodies (62.4 % women, mean age 46.8 years) who received confirmatory RT-PCR testing for viremia (63.2 % HCV-RNA-positive).

Results: twenty-two patients had T2DM (17.6 %, 95 % confidence interval: 11.8-25.3 %; mean National prevalence: 14.4 %), more frequent among patients with detectable viremia than in negative cases (23.3 % *vs.* 9.6 %, respectively; *p* = 0.04), and among those with advanced liver disease, than in compensated patients (28.9 % *vs.* 11.3 %, respectively; *p* = 0.01). Fourteen (17.7 %) patients received interferon-based treatment and 6 (42.8 %) had sustained virology response. None of the 6 responders had T2DM, but 2 of the 8 (25 %) non-responders had diabetes. T2DM patients were older than those without diabetes (57.7 *vs.* 44.5 years, *p* < 0.001), and after multivariate analysis, only age was significantly associated with diagnosis of T2DM.

Conclusions: T2DM was highly prevalent among patients with chronic HCV infection. Age was the most important determining factor.

Key words
diabetes mellitus, type 2
hepatitis C
liver

Diabetes mellitus (DM) and chronic liver disease are highly prevalent in Mexico, they represented the first and fourth cause of general mortality in 2008, respectively.[1] The relationship between several liver diseases and abnormalities of glucose metabolism has been recognized since antiquity and classical medical textbooks.[2,3] Modern medicine has also demonstrated the magnitude of this association, with salient clinical implications in diagnosis, treatment and prognosis.[4-6]

Near 40 % of patients with the hepatitis *C* virus (HCV) infection have extrahepatic manifestations of the disease.[7] A considerable body of clinical and experimental evidence has consistently indicated a link between HCV infection and type 2 DM (T2DM).[8-21] It appears that this relationship goes beyond the mere liver injury as was first hypothesized.[8] Since chronic HCV infection implies a state of systemic inflammation that leads to peripheral, rather than hepatic, insulin resistance.[20] Moreover, HCV can infect the pancreatic β-cell and

potentially impair its endocrine function.[21] According to the most recent data, prevalence of diabetes mellitus in Mexican adults is 14.4 %.[22] Frequency of confirmed cases living with HCV infection in Mexico is estimated to be < 1 %.[23,24] Surprisingly, data on the association of HCV infection with T2DM in Mexico are scarce.[5,6] We aimed to test the hypothesis that HCV infection is associated with a high prevalence of T2DM in Mexican adults.

Methods

A retrospective study was performed on a research database of 125 consecutive patients aged > 20 years, all reactive to anti-HCV antibodies, who were referred for molecular testing and confirmation of HCV infection, to the Department of Molecular Biology at the Hospital Civil de Guadalajara "Fray Antonio Alcalde". The internal Committee of Ethics of our hospital approved the study. Informed consent was required to be included in our research database.

Ambulatory patients were referred from blood bank and infectology, gastroenterology, general internal medicine, pediatrics and hematology departments. We considered for this study only adult patients reactive to a screening test for anti-HCV antibodies in at least two occasions, with or without clinical manifestations of liver decompensation. A standardized structured questionnaire was used to collect data from the patient regarding demography, relevant antecedents and risk factors. Overt liver decompensation was considered to occur in a patient if jaundice, ascites or collateral superficial veins were present. The antecedent diagnosis or previous hospitalization for liver decompensation (i.e., upper gastrointestinal bleeding, hepatic encephalopathy, and other complications) was also considered as liver decompensation.[25] Diagnosis of diabetes was registered by history taking and fasting blood glucose $\geq$ 126 mg/dL in at least two occasions.[21] Cases with type 1 diabetes mellitus were not included in the present analysis.

A 9 mL blood sample was obtained in 3 test tubes (3 mL each) from a peripheral vein for serologic and molecular assays. An automated third generation microparticle enzyme immunoassay (MEIA, IMx HCV Version 3.0 Abbott Diagnostics, Chicago, IL, USA) was used to assess the presence of anti-HCV antibodies in sera samples stored at –70 ºC.[25] Immunoassay signal strength of the sample to cut-off rate (S/CO) ratio > 1 was considered as reactive test, according to directions of the
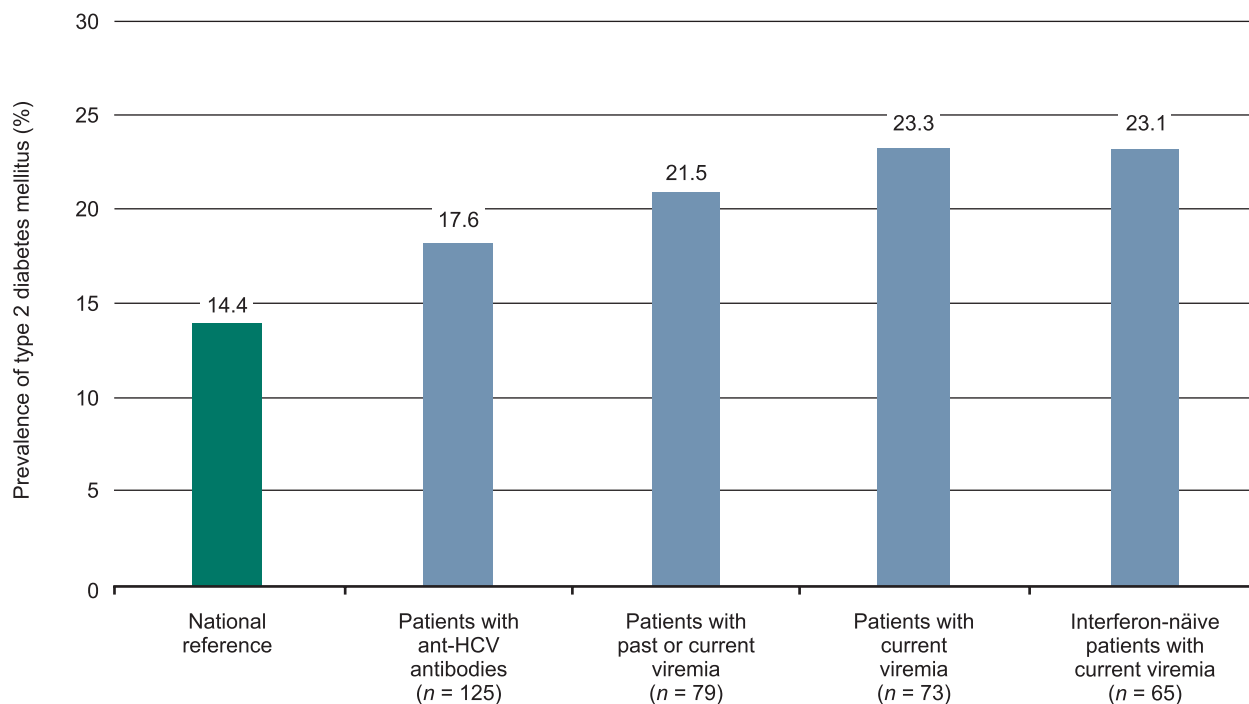

Figure 1 | Type 2 diabetes mellitus among different subgroups of patients, as compared with the Mexican prevalence. The shadow background emphasizes the difference between subgroups of viremic patients and the reference standard, as well as the putative protective effect of the interferon-based therapy

manufacturer. A home-made qualitative nested reverse-transcription polymerase chain reaction (RT-PCR) was used to detect HCV RNA in all sera samples, first stored at –70 ºC. Total RNA was extracted from each serum without pooling, using QIAamp Viral RNA Mini Kit (QIAGEN, Chatsworth, CA) as indicated the manufacturer. Then, RT was carried out to obtain complementary DNA (cDNA) using M-MLV RT kit (MMLV, GIBCO/BRL). PCR amplification of cDNA and later a nested-PCR were performed with two pairs of primers that hybridize in a segment of the 5' non-coding region of the HCV genome, as is described elsewhere.[25-27] Nested RT-PCR was performed per duplicate in all samples and the reaction products were analyzed in gel electrophoresis.

Statistical analysis

Demographic data are reported as simple frequencies. The 95 % confidence interval for relevant frequencies was calculated with the adjusted Wald method. Age and S/CO ratios are presented and analyzed as medians with minimum and maximum, as these variables followed a non-normal distribution. Chi-square or Fisher exact test was used for nominal variables in univariate analyses, as indicated. Student *t* test was used to compare continuous, normally distributed variables. Mann-Whitney *U* test was performed when an ordinal or scale non-parametric variable was distributed between two groups. To find independent predictors for the presence of diabetes mellitus, multivariate analyses were performed by forward stepwise logistic regression. Adjusted odds ratios (OR) with 95 % confidence intervals (CI) are provided. The fitness of the model was evaluated by using the Hosmer-Lemeshow goodness-of-fit test, which was considered as reliable if *p* was > 0.2. Statistical comparisons or interactions with $p < 0.05$ were regarded as significant. All *p* values reported are two-sided. SPSS v. 17.0 (Chicago, Ill; USA) statistical package was used for all the analyses.

Results

We studied 125 patients reactive to anti-HCV antibodies in at least two occasions: 78 (62.4 %) women and 47 (37.6 %) men. After inclusion, all patients received at least two qualitative RT-PCR assays for confirmation of HCV viremia, and those suitable for antiviral treatment received a quantitative assessment for viral load and genotyping. Mean age was 46.8 years (range 21 to 82 years). There was only one patient with HBV infection, confirmed by PCR testing, who had a negative RT-PCR test for HCV. Of the 125 patients studied, 46 (36.8 %) were false-positive cases (with low, although positive signal in anti-HCV serology), with a repeatedly negative RT-PCR for HCV-RNA. On the other hand, 79 (63.2 %) patients had confirmed HCV infection by RT-PCR testing, and of them,

14 (17.7 %, all with HCV 1a/1b genotypes) received interferon-based treatment, with 6 (42.8 %) patients who had cleared the virus by the time they were re-assayed for HCV viremia. As a result, 73 (58.4 %) of the 125 patients had a positive test for HCV viremia in our laboratory, which includes 8 non-responders to interferon-based therapy. Mean age of the confirmed cases was higher than that of the HCV-negative cases (49.2 *vs.* 42.6 years, respectively; $p = 0.02$). Age was not significantly different between patients who received interferon therapy and non-treated patients (48.2 *vs.* 49.4 years; respectively; $p = 0.74$).

In all, the frequency of T2DM was 17.6 % (95 % confidence interval: 11.8–25.3 %; $n = 22$), which was significantly more prevalent among patients with HCV viremia, than in patients with no detectable HCV RNA in serum (23.3 % *vs.* 9.6 %, respectively; $p = 0.04$). None (0 %) of the 6 responders (either with early or sustained viral response) to interferon-based therapy had T2DM, as compared with 2 (25 %) among the 8 non-responders). Patients with T2DM were significantly older than patients without diabetes (57.7 *vs.* 44.5 years, respectively; $p < 0.001$). Nevertheless, T2DM appeared to be more prevalent in HCV-infected patients than the National Mexican reference of 14.4 % (figure 1), especially for the age groups 50 to 59 and 60 to 69 years (table I). After multivariate analysis, controlled for viral status, among other variables, only age was significantly associated with T2DM (table II). Advanced liver disease (i.e., liver decompensation) was observed in 45 patients, who were significantly older than patients without decompensated liver disease (51.4 *vs.* 44.2 years, respectively; $p = 0.002$). T2DM was significantly more prevalent among patients with advanced liver disease, than in compensated patients (28.9 % *vs.* 11.3 %, respectively; $p = 0.013$).

Discussion

In the present study, patients who had HCV viremia, either because they had not received or did not respond to interferon-based therapy, had a high frequency of T2DM. Our study was underpowered to detect a significant difference on T2DM frequency between responders and non-responders to interferon-based treatment; however, it has been previously noted that the risk of T2DM lowers after clearing the virus.[19,28] This represented an issue deserving in-depth study in Mexico and Latin America. Chronic HCV infection enables a state of inflammation, post-insulin receptor (i.e., type D) insulin resistance and impaired β-cell function.[20,21,29,30] These factors together, impaired insulin secretion and action, represent the condition *sine qua non* T2DM occurs. But the reason that most HCV-infected patients (usually > 70 %) actually will not develop diabetes, is a very interesting and yet unresolved issue that may be more related to host factors than to viral characteristics.[4] It represents a potential

Table I | Prevalence of type 2 diabetes mellitus across age groups, as compared with the Mexican standard reference (Ensanut 2006)[22]

Age decades	National Mexican prevalence %	All patients reactive to antiHCV antibodies (*n* = 125) %	Patients with confirmed past or present viremia (*n* = 79) %	Patients with current viremia (*n* = 73) %	Interferon-näive patients with current viremia (*n* = 65) %
20-29	3.3	0	0	0	0
30-39	8.5	6.3	0	0	0
40-49	16.5	3.0	4.0	4.2	4.5
50-59	31.1	32.4	40.0	47.6	44.4
60-69	32.75	46.7	50.0	50.0	55.6
70-97	26.1	20.0	25.0	25.0	25.0

area of research in molecular biology and genomics applied to medicine.

The present report provides relevant clinical information and hypotheses with potential issues to pursue. This study, although exploratory in its nature, is different from most publications about this issue in that only patients reactive to anti-HCV antibodies were analyzed, and comparators (i.e., controls) of the frequency of T2DM were non-viral patients within the same seropositive population, and still, an association was found between HCV infection and T2DM. In most previous epidemiological studies, although large, do not distinguish between seropositive patients to anti-HCV antibodies and true infected persons with detectable viremia by RT-PCR, and in some reports controls are healthy individuals negative to anti-HCV antibodies.[8-21] It is difficult to assess the pathophysiological relationship between HCV infection and altered glucose metabolism when it is not clear how many seropositive patients to anti-HCV antibodies are in fact infected with HCV, if no confirmatory tests are performed by molecular assays. The rate of false-positives can be high in certain populations.[24,25] This factor may account, at least in part, for the wide variation on the frequency of T2DM in HCV-infected individuals across studies. Interestingly, in the present report the frequency of T2DM among HCV-infected patients is quite similar ($\approx$ 22 %) to that reported in previous Mexican studies addressing a similar topic.[5,6] However, in our report patients with diabetes were older than those without this condition, which may confound the magnitude of the association. This particular issue will only be clarified with a sufficiently powered, large-scale community-based study. Nonetheless, as compared with the national reference, frequency of T2DM seems to be consistently higher in the subgroups of patients with either past or current HCV viremia. Furthermore, as it has been described for the US population.[11] The older patients presented in this paper had the highest difference in diabetes frequency, when compared with the population reference within the same age group.

Table II | Multivariate analysis on factors associated with type 2 diabetes mellitus: a binary logistic regression model.*

Variable	Regression coefficient	Standard error	Odds ratio (95 % confidence interval)	*p*
Age, each decade from 20 years	0.86	0.24	2.36 (1.47-3.79)	< 0.001
Constant	−5.60	4.328	—	< 0.001

*Adjusted for gender, HCV viremia, liver decompensation, alcohol consumption, smoking habit and educational level. Hosmer-Lemeshow test for goodness of fit in final step of the regression model: $\chi^2 = 4.37$, 1 df, $p = 0.22$

Although clearly underpowered to detect small, but clinically meaningful differences, this study represented the largest focusing exclusively on patients reactive to anti-HCV antibodies in Mexico.[5,6] Nonetheless, important limitations should be emphasized. The design of this study could not account for several variables known to be associated with T2DM, among the most important, body mass index, adiposity, diet, physical activity, family history and several biomarkers. Moreover, the exact proportion of patients with cirrhosis could not be estimated by means of liver biopsy. Hence, this study should be interpreted as hypotheses generating, which warrants further research in the Mexican population.

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