GLYCEMIC HOMEOSTASIS IN CHRONIC VIRAL HEPATITIS AND LIVER CIRRHOSIS

N. CUSTRO, A. CARROCCIO, A. GANCI, V. SCAFIDI, P. CAMPAGNA, L. DI PRIMA, G. MONTALTO

SUMMARY - Objectives: This study aimed at investigating the respective impacts of virus-related chronic hepatitis (CH) and liver cirrhosis (LC) on glycemic homeostasis, with reference to grading and/or staging of liver disease and to contribution of the two main responsible viruses.

Material and methods: The glycometabolic features of 82 patients with CH (B-related 16, and C-related 66) and 145 with LC (B-related 24, and C-related 121) were evaluated.

Results: Impaired glucose tolerance (IGT) was detected in 9 (11.0%) and diabetes mellitus (DM) in 6 (7.3%) of the CH patients [(P < 0.05 vs controls, in both cases; respective odds ratios (95% CI): 2.6 (1.1-6.3), and 4.0 (1.2-13.2)]. IGT was detected in 86 (59.3%) and DM in 34 (23.4%) of the LC patients [(P = 0.000 vs controls, in both cases; respective odds ratios: 10.0 (7.0-14.4), and 5.5 (3.5-8.5)]. The odds ratios for the prevalence of IGT and DM in the LC patients were 11.8 (5.2-27.5) and 3.9 (1.5-10.8), compared with the CH patients. In the CH patients, glycometabolic failure was significantly related to age (P = 0.026), but not to grading and staging, and in the LC patients to Pugh-Child score (P = 0.037). IGT was found in 17/40 (42.5%) HBV-related patients and in 13/40 (32.5) matched HCV-related patients. DM was found in 9/40 (22.5%) HBV-related patients and in 10/40 (25.0%) HCV-related matched patients, without significant difference in the respective proportions.

Conclusion: The prevalence of DM associated to virus-related CH is on average four times higher than in the general population, independently of the histopathological picture of disease. Virus-related LC further increases the prevalence of both IGT and DM, independently of sex and age, but in relationship with the severity of disease. HBV and HCV infections do not appear to have a different impact on glycemic homeostasis.

Key-words: diabetes mellitus, impaired glucose tolerance, chronic hepatitis, cirrhosis.

RESUME - Homéostasie glycémique au cours de l’hépatite virale chronique et de la cirrhose hépatique.

Objectifs : Cette étude vise à analyser l’impact respectif des hépatites virales chroniques (CH) et de la cirrhose hépatique (LC) sur l’homéostasie glycémique, en rapport avec la stadiification de la maladie hépatique et avec la contribution des deux principaux virus responsables.

Matériel et méthodes : Les caractéristiques du métabolisme glucidique de 82 patients avec CH (HBV 16, HCV 66) et 145 avec LC (HBV 24, HCV 121) ont été analysées.

Résultats : Une intolérance au glucose (IG) a été retrouvée chez 9 (11.0 %) et un diabète (DM) chez 6 (7.3 %) des patients CH [(P < 0.05 vs témoins, dans chaque cas ; odds ratios respectif (95 % CI) : 2.6 (1.1-6.3), et 4.0 (1.2-13.2)]. IG a été détectée chez 86 (59.3%) et DM chez 34 (23.4%) des patients LC [(P = 0.000 vs témoins, dans chaque cas ; odds ratios respectif : 10.0 (7.0-14.4), et 5.5 (3.5-8.5)]. Les odds ratios pour la prévalence de l’IG et du DM chez les patients étaient de 11.8 (5.2-27.5) et 3.9 (1.5-10.8), comparés aux patients CH. Chez les patients CH, les anomalies glucidiques étaient significativement liées à l’âge (P = 0.026), pas mais au grade de l’hépatopathie, et chez les patients LC au score de Pugh-Child (P = 0.037). IG a été notée chez 9/40 (22.5 %) et un DM chez 10/40 (25,0 %) des patients LC [(P = 0,000 vs témoins, dans chaque cas ; odds ratios respectif : 10,0 (7,0-14,4), et 5,5 (3,5-8,5)]. Les odds ratios pour la prévalence de l’IG et du DM chez les patients étaient de 11,8 (5,2-27,5) et 4,9 (1,3-10,8), comparés aux patients CH. Chez les patients CH, les anomalies glucidiques étaient significativement liées à l’âge (P = 0,026), pas mais au grade de l’hépatopathie, et chez les patients LC au score de Pugh-Child (P = 0,037). L’IG était notée chez 17/40 (42,5) % des patients HBV et chez 13/40 (32,5) % des patients HCV apparus. Le DM était trouvé chez 9/40 (22,5 %) des patients HBV et 10/40 (25,0 %) des patients HCV apparus, sans différence significative.

Conclusion : La prévalence du diabète associé aux hépatites virales est en moyenne 4 fois plus forte que dans la population générale, indépendamment de l’aspect histologique de la maladie. La cirrhose post-virale augmente encore la prévalence de l’IG et du DM, indépendamment du sexe et de l’âge, mais en relation avec la sévérité de la maladie. L’infection par HBV ou HCV n’apparaît pas avoir un impact différent sur l’homéostasie glycémique.

Mots-clés : diabète, intolérance au glucose, hépatite chronique, cirrhose.
Adequate liver function is known to be essential for carbohydrate metabolism [1]. Thus, it is not surprising that abnormalities in glycemic homeostasis have already been recognized as a possible complication of chronic liver disease [2-4]. However, while recent investigations have concentrated on the mechanisms underlying this association, clinical studies in the literature have often been uncontrolled and in any case have shed limited or only indirect light on the presumably different impacts that histologically and/or clinically different types of chronic liver disease may have on glycemic homeostasis. Moreover, a previous retrospective review of cirrhotic patients awaiting liver transplantation, which suggested a close link between hepatitis C virus (HCV) infection and overt diabetes mellitus [5], has emphasized the impact that single viruses could have on the liver-dependent impairment of glucose metabolism, and has increased the need for further data.

We systematically assessed the glycometabolic characteristics of patients with virus-related chronic liver disease seen in our Unit over the years 1993/98. The aim of this work was to estimate: a) the prevalence of both minor (impaired glucose tolerance) and major (diabetes mellitus) glycometabolic failure in these patients, compared to the general population; b) the connection – if any – between severity and progression of liver disease and glucose intolerance; and, finally, c) the contribution of two main responsible viruses.

**PATIENTS AND METHODS**

**Patients**

Between October 1992 and September 1998, we considered for this study all patients with chronic virus-related liver disease observed in our Unit, with the following exclusion criteria: pre-existing conditions predisposing to hyperglycemia, including current use of alcohol or drugs known to affect blood glucose levels; past treatment with interferon; coincidental presence of hepatocarcinoma: glycometabolic alterations preceding the appearance of hypertransaminasemia. Three hundred and sixty-six patients were eligible, but we were only able to assess glucose metabolism after a complete liver evaluation, including histology when necessary to diagnosis, in 227 (M 127 and F 100), because 139 patients did not consent to the study or dropped out of the follow-up.

The patients were classified as having chronic hepatitis (CH) or liver cirrhosis (LC) in accordance with the histopathological criteria of Sherlock [6]. The patients who had a reliable diagnosis of LC according to clinical, biochemical and instrumental data, did not undergo liver biopsy and were classified according to the Pugh-Child score [7].

As controls, we used 3405 consecutive subjects negative for biochemical function tests of liver disease, taken from the screening performed on the local general population in the same time interval as the study. The control subjects were subdivided into two subgroups of 415 and 2990 subjects on the basis of an age- and sex-matching with the two groups of patients (the first presenting CH and the second LC, respectively).

Informed consent was obtained from all patients after approval of the protocols by the Human Investigation Committee in our hospital, and the study was carried out according to the principles expressed in the Declaration of Helsinki.

**Methods**

**Serology**

HBV serological markers were tested using commercially available kits (Abbott Laboratories, Chicago, IL). Anti-HCV antibodies were assayed using the second generation enzyme-linked immunosorbent assay (ELISA; Ortho Diagnostic System, Raritan, NJ) and positive samples were confirmed by immunoblotting (RIBA II, Chiron Corporation, Emeryville, CA).

**Histopathological Analysis**

Specimens were obtained percutaneously with a Menghini needle. All samples were stained with hematoxylin and eosin, Perls blue, Prussian blue orcein and examined by the same pathologist, who was unaware of the clinical, biochemical and instrumental data. The histological score of CH was calculated according to the score of Desmet et al. [8], which distinguishes between grading (score 0-18) obtained by the sum of histological changes expressed by Knodell’s numerical scoring system [9] (periportal necrosis, intralobular degeneration and portal inflammation), and staging, an expression of fibrosis (score 0-4).

**Glycemic assessment**

Anamnestic data were first obtained from each patient, including the time of discovery of hyperglycemia – if any – and the hypoglycemic treatment. Plasma glucose levels after an overnight fast (FPG) was assessed in all recruited patients. If higher than 6.0 mmol/l, determination was confirmed twice and: – a) when between 6.1 and 6.9 mmol/l, a standardized oral glucose tolerance test (OGTT) was performed and evaluated using the 2-h plasma glucose level in accordance with 1985 WHO criteria [10]; – b) when higher than 6.9 mmol/l, the patient was considered diabetic, as well as those already on glucose-lowering treatment, and not submitted to OGTT. Glycemic assess-
ment of the control subjects was performed with the same methodology. Plasma glucose concentrations were measured by the glucose-oxidase method on a Beckman Glucose Analyser II (Beckman Instruments, Fullerton, CA). The analyser was monitored monthly in our clinical centre. The values were within 0.1 mmol/l of those obtained by an external quality assessment scheme.

**Statistics**

Values are expressed as mean ± SD, as median with range (min-max), and as percentages. Comparisons of proportions in the single groups and subgroups of patients were performed using the Z-test. Odds ratios and 95% confidence interval (CI) were also calculated. Correlation between glycometabolic condition and age, grading and staging of CH, or clinical scoring of LC was assessed with the non-parametric Kendall’s rank-test. Analyses were performed using StatView Software (Abacus Concepts, Berkeley, CA).

**RESULTS**

The general, biological, histopathological and clinical characteristics of the 227 patients we assessed (divided according to liver disease) and of the 3405 in the control group are summarized in Table I. The sex-ratios in the groups of patients were markedly different. Moreover, as expected, the age of the LC patients was significantly higher then the age of the CH patients (P = 0.000).

Table II shows the prevalence of glycemic alterations in patients grouped according to the type of liver disease and to the responsible viruses. Overall, 15 of the 82 CH patients (18.3%) had one of the two alterations in glycemic homeostasis. The prevalence of IGT was significantly greater than in the sex- and age-matched controls: difference (d) = 0.06 (with the 95% confidence interval, 0.01-0.12), P = 0.042; odds ratio 2.6 (with 95% CI = 1.1-6.3) V. The prevalence of DM in the same group was also significantly higher: d = 0.05 (0.02-0.09), P = 0.019; odds ratio 4.0 (1.2-13.2) V. The prevalence of all types of glycometabolic failure in the CH patients was significantly higher than in the controls: d = 0.12 (0.05-018), P = 0.000.

In the LC patients, the overall prevalence of glycometabolic failure was even more higher (82.7%) than in the sex- and age-matched controls (18%): d = 0.65 (0.58-0.72), P = 0.000. In detail: for IGT, d = 0.47 (0.41-0.53), P = 0.000; for DM d = 0.18 (0.14-0.22), P = 0.000. Odds ratios for the prevalences of IGT and DM were 10.0 (7.0-14.4) and 5.5 (3.5-8.5), respectively.

The prevalence of IGT and DM in the LC patients was significantly higher than in those with CH: for IGT, d = 0.48 (0.35-0.61), P = 0.000, odds ratio 11.8 (5.2-27.5); for DM, d = 0.16 (0.05-026), P = 0.004, odds ratio 3.9 (1.5-10.8). The prevalence of all types of glycometabolic failure in the LC patients was significantly higher than in the CH patients: d = 0.64 (0.51-0.77), P = 0.000. The M/F ratio in both IGT and DM patients was similar to that of the single groups. In detail: the overall M/F ratio was 54/28 in the whole CH patient group, 6/3 in the IGT cases and 4/2 in the DM cases; moreover, the overall M/F ratio was 73/72 in the whole LC patient group, 42/44 in the IGT cases and 18/16 in the DM cases.

In Table III, we reported data regarding the prevalence of glycemic alterations with reference to the contribution of two single viruses. There were no statistically significant differences in any comparison.

<table>
<thead>
<tr>
<th>Table I. Characteristics of patients and controls in the study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CH patients</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Subgroup I</strong></td>
</tr>
<tr>
<td>M/F</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Staging</td>
</tr>
<tr>
<td>Grading</td>
</tr>
<tr>
<td>Pugh-Child</td>
</tr>
</tbody>
</table>

CH = Chronic Hepatitis; LC = Liver Cirrhosis (Plus-minus values are means ± SD).
Moreover, in a subgroup of 40 out of the 187 C-related patients matched for sex, age and stage of liver disease with the 40 B-related patients, there was no significant difference in the proportion of IGT and DM.

<table>
<thead>
<tr>
<th></th>
<th>CH patients (n = 82)</th>
<th>Controls (n = 415)</th>
<th>P value</th>
<th>Odds ratio (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGT</td>
<td>9 (11.0 %)</td>
<td>19 (4.6 %)</td>
<td>0.042</td>
<td>2.6 (1.1-6.3)</td>
</tr>
<tr>
<td>DM</td>
<td>6 (7.3 %)</td>
<td>8 (1.9 %)</td>
<td>0.019</td>
<td>4.0 (1.2-13.2)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (18.3 %)</td>
<td>27 (6.5 %)</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

In the CH patients, the presence and degree of glycometabolic alterations were well-correlated with age (T = 0.17 and Z = 2.23; P = 0.026), but not with liver disease grading (T = 0.02 and Z = 0.32; P = 0.745) and staging (T = 0.04 and Z = 0.52; P = 0.599). In contrast, in the LC patients the presence and degree of glycometabolic alterations were not related to patient’s age (T = 2.68*E^-3 and Z = 0.05; P = 0.962), but to the Pugh-Child score (T = 0.12 and Z = 2.08; P = 0.037).

**DISCUSSION**

The results of this study refer to a group of patients, the composition of which reflects the epidemiology and the natural history of chronic liver disease as it can be observed in our area. In particular, there is a considerably higher incidence of C-related than B-related disease, a higher prevalence in males of non-cirrhotic chronic liver disease, while in the cirrhotic stage incidence tends to be equivalent in both sexes, as though the illness in males (in the absence of other factors, especially alcohol) follows a less aggressive evolution. Finally, and obviously, the mean age of cirrhotic patients is higher.

Bearing this in mind, our investigation found prevalences of glycometabolic alterations in patients with virus-related chronic liver disease four times higher, on average, than those in the general population of the same age. Previous data regarding the
prevalence of glycometabolic alterations in patients with chronic liver disease [2-4, 11-15] have been not only contradictory but also difficult to compare with our results. Clearly, the proportion of patients with overt LC in the different surveys had a considerable weight on the respective results; moreover, patients with alcoholic cirrhosis and/or with hepatocarcinoma were often included in some investigations; finally, cases with DM identified before hepatopathy were also considered. Our results, obtained only from patients with virus-related chronic liver disease and glycemcic abnormalities subsequent to the finding of liver disease, indicated that more than 40% of all patients had IGT (vs 11.7% in the general population of the same age in our area), and more than 17% had DM (vs 4.9%).

Although previous studies have all reported a frequent association, especially between LC and DM, very few have provided selective data regarding the presumably lower impact that CH, which has not yet evolved to cirrhosis, has on glycemic metabolism [16]. In the majority of the investigations, in fact, data related to glycometabolic alterations in patients with liver disease still at the CH stage have been mixed with ones which mostly concern cirrhotic patients [3-5, 11-13]. We demonstrated the existence of a perceptible trend towards glucose intolerance in patients not yet cirrhotic. The proportion of patients we found with IGT indicated a more than 2-fold additional risk associated with CH, and the proportion of patients with DM indicated a risk 4-fold greater than in individuals without liver disease. However, the relative frequency of IGT in patients still at the CH stage is approximately 1/6 and the relative frequency of DM is approximately 1/3 of that documented in overtly cirrhotic patients.

This study indicated that the probability of developing glycometabolic alterations in both the CH and LC patients is independent of sex, since the proportion of affected individuals reflect the composition of the two groups. In contrast, this probability could increase with age, as in the general population, since there is a higher prevalence in cirrhotic patients, who naturally tend to be older.

This investigation failed to demonstrate the existence of a clear correlation between inflammatory alterations in the liver, as assessed by histology, and glycemic abnormalities. This negative finding might suggest that, at least until LC develops, glycemic imbalance would be more likely to be due to patient age and to pre-existing genetic and/or acquired environmental factors, whereas liver impairment could accelerate the process. One alternative hypothesis could be that histological characteristics do not sufficiently reflect liver function capacity in patient with CH.

This study, instead, provides further evidence regarding the considerably increased risk of glycometabolic failure associated with functional liver decompensation, typical of LC patients. Unlike the CH patients, however, in the LC patients the risk of a major or minor deficit in glucose metabolism proved to be independent of age. At this stage, the correlation between the general clinical feature (as revealed by the Pugh-Child score) and the risk of an altered glycemic profile is meaningful. These findings are easily explained in the light of what is known about the mechanisms of glycometabolic failure in patients with liver disease [17, 18]. In fact, it is to be expected that liver glucose uptake and peripheral insulin sensitivity should decrease partly with age, but mainly with the severity of liver function impairment.

The presumed existence of a preferential link between HCV infection and glycometabolic failure has been previously emphasized [5, 19]. Further contributions to this thesis, indeed, appeared in the literature while the present work was in progress [20, 21]. Our investigation failed to support the hypothesis that one of the two main viruses responsible for chronic liver disease might be associated with either IGT or DM. Allison et al., who first claimed this association [5], retrospectively evaluated data regarding only seriously ill patients with established cirrhosis, candidates for liver transplantation. HCV is notoriously responsible for the most unfavourable cases of chronic liver disease [22], thus it cannot be excluded that their conclusion may depend on the characteristics of the particular patients reviewed. Furthermore, in the work of Fraser et al. [19], the higher mean age of the patients with HCV infection, in comparison with HBV-related patients, might have pointed to a HCV-DM association, because in that investigation age was found to be another independent predictor for developing diabetes, which we only found in patients with CH. Likewise Mason et al. [20], who also pointed to a relatively strong association between HCV infection and diabetes, concluded that HCV can be seen as a further risk factor, in addition to that attributable to chronic liver disease per se, especially in patients with genotype 2a. Also from the work of Caronia et al. [21] the weight of the stage of chronic liver disease clearly rises, since cirrhosis was found to be the major variable associated with DM.

The limited availability of patients with B-related chronic liver disease unquestionably suggests the need for caution in reaching a conclusion in this matter and large meta-analysis would be required. Thus, it is not surprising that also in other recent investigations an association between HCV infection and glucose intolerance has been refuted [23, 24].

In summary, virus-related chronic liver disease has a considerable impact on blood glucose homeostasis. This impact, which is mild in CH patients, remarkably increases in patients with overt LC in parallel with a worsening of the clinical picture. We did not prove that a specific type of virus responsible for chronic liver disease might represent a greater impact factor.
for glycometabolic failure. The findings of this investigation seem to suggest that the increased frequency of glycemic imbalance is only related to the degree of liver function impairment, independent of its nature.

Acknowledgement — This investigation was supported in part by a 60% grant for MURST (1993, 1997).

REFERENCES