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Original Article

Insulin resistance in chronic hepatitis B and C

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Aim: To determine whether insulin resistance occurs in patients with chronic hepatitis B (CHB) and chronic hepatitis C (CHC), and its relationship with the presence of liver fibrosis and steatosis. **Methods:** Untreated patients with CHC (n=60) or CHB (n=40), similar in age, gender, body mass index and waist-hip ratio, were studied. Relationship between anthropometric, biochemical (fasting serum insulin, C-peptide, ferritin, iron, TNF-α, cholesterol, triglyceride, bilirubin, hemoglobin, and platelet concentrations) and liver biopsy (43 CHC and 20 CHB patients) findings was investigated by insulin resistance determined via the homeostasis model assessment (HOMA-IR). **Results:** The mean fasting serum insulin was 14.9 (11.9) mU/mL in CHC and 21.4 (17.4) in the CHB group (normal range 0.7-9; p=0.049) and mean HOMA-IR was 3.1 (2.6) in CHC versus 4.7 (4.1) in the CHB group (normal range 0.12-4.61; p=0.036) . HOMA-IR was significantly associated with fibrosis stage in the CHC group (p=0.015), but not in the CHB group. **Conclusion:** Hyperinsulinemia occurs in chronic viral hepatitis B and hepatitis C; insulin resistance is associated with stage of fibrosis in hepatitis C. [*Indian J Gastroenterol* 2006;25:286-289]

Recent evidence suggests that chronic hepatitis C (CHC) is associated with increased risk of development of type II diabetes, irrespective of the presence of cirrhosis.[1] Insulin resistance (IR) plays a primary role in this development. Prospective longitudinal studies have shown that IR precedes the onset of diabetes by 10-20 years.[1] Also, the presence of diabetes may be associated with an increased rate of progression of fibrosis in patients with CHC and chronic hepatitis B (CHB).[1]

We tested the hypothesis that CHC may lead to IR, and studied the relation between IR and anthropometric, biochemical, hematological and histological findings and iron deposits. We hypothesized that iron stores in liver tissue and increased serum TNF-α level could explain this association.

Methods

The study included 60 patients with CHC (positive for serum anti-HCV using a third-generation enzyme immunoassay and for HCV RNA using a quantitative or a qualitative HCV RNA assay) and 40 patients with chronic hepatitis B (CHB; positive for HBsAg) with elevated serum transaminase levels for at least 6 months, attending the hepatitis clinic between September 2003 and April 2005. Exclusion criteria were: prior antiviral treatment, established diabetes, concurrent hepatitis B and C virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, hemochromatosis, α1-antitrypsin deficiency, Wilson's disease, patients receiving drugs or having conditions that cause fatty liver (tamoxifen, steroids, amiodarone, diltiazem, gastrointestinal bypass surgery, or severe recent weight loss), regular or excessive alcohol consumption, pregnancy and lactation. No patient had evidence of hepatic decompensation (hepatic encephalopathy, ascites, variceal bleeding, or serum bilirubin level >2-fold the upper limit of normal).

Liver biopsy was done only if there was a definite indication and patients consented. It was considered adequate if it included at least 4 portal tracts. An experienced pathologist, who was unaware of the clinical data, scored the biopsy for grade (scale 0-24) and stage (scale 0-6), using the modified histological activity index (HAI).[2,3] Steatosis was graded according to the proportion of hepatocytes containing macrovesicular fat droplets (grade 0: no steatosis, 1: <33% hepatocytes, 2: 33%-66%, and 3: >66%).[1]

Iron stores were graded as follows:[4] 0 – no or barely discernible granules at 400x magnification; 1 – barely discernible at 250x but easily confirmed at 400x, 2 – discrete granules resolved at 400x; 3 – discrete granules resolved at 25x; and 4 – masses visible at 10x or naked eye.

Clinical and laboratory assessment

At enrollment, age, gender, weight, height, body mass index (BMI), waist-hip ratio (WHR), average daily

alcohol consumption (g/day), opium use, drug history including intake of iron supplements, and family history of diabetes were recorded. HCV genotype was identified using a second-generation reverse hybridization line probe assay (Bayer, Tarrytown, New York; manufactured by Innogenetics, Ghent, Belgium).

After an overnight fast, blood was drawn from an antecubital vein into a 10 mL tube. It was centrifuged at 2500 g for 15 min within 1 hour of collection, and serum was stored at -80°C till analysis. Serum levels of triglycerides, total cholesterol, HDL cholesterol and glucose were measured using enzymatic methods (Pars Azmoon, Tehran, Iran) and an auto-analyzer (Vita Lab Selectra 2, Helsinki, Finland). Concentration of low-density lipoprotein cholesterol was calculated using Friedewal's formula.[5] Intra-assay coefficients of variation for triglyceride, total cholesterol and HDL-C were less than 5.2%. Iron concentration was determined by chemical colorimetric method (Pars Azmoon, Tehran, Iran); intra-assay coefficient of variation was 4.9%. Enzyme immunoassays were used to measure serum TNF-α (Bendermed, Vienna, Austria), and insulin and C-peptide (Monobind, Lake Forest, USA) levels.

IR was calculated using the homeostasis model assessment (HOMA) method as per the following equation: insulin resistance (HOMA-IR) = fasting insulin (μU/mL) x fasting glucose (mmol/L)/22.5.

The study protocol was approved by our hospital's Ethics Committee and all patients gave written informed consent.

Statistical analysis

Univariate methods included *t* test for continuous variables, and chi-squared or Fisher's exact test for categorical variables. For multivariate analysis, multiple linear regression was used. SPSS software version 11.0 was used, and *p* values <0.05 were considered significant.

Results

The two groups (CHC and CHB) were similar in age (mean [SD]: 36.6 [11.1] years *vs* 36.3 [13.4]), gender (males: 50/60 *vs* 32/40), BMI (23.9 [4.2] *vs* 25.2 [3.9]) and WHR (0.83 [0.82] *vs* 0.99 [0.12]). Patients with CHC more often reported opium (19/60 [32%] *vs* 2/40 [5%; p=0.001] and alcohol use up to 15 g/d (24/60 [40%] *vs* 8/40 [20%; p=0.049]) than those with CHB. History of diabetes in first-degree relatives was more common in CHC (5 [8%] than in CHB (6 [15%]; p=ns).

Table 1: Laboratory findings in patients with chronic hepatitis C (CHC) and chronic hepatitis B (CHB)

Characteristics	CHC (n=60)	CHB (n=40)	*p*
Hemoglobin (g/dL)	13.5 (2.5)	12.8 (1.9)	0.31
Platelet count (x10^3 cells/mm^3)	197 (70)	190 (90)	0.68
ALT level (U/L)	76.9 (57.0)	81.3 (89.6)	0.77
AST level (U/L)	70 (59)	81 (149)	0.68
Bilirubin (mg/dL)	2.4 (9.0)	2.2 (4.6)	0.90
INR	1.2 (0.3)	1.2 (0.5)	0.85
Fasting sugar (mg/dL)	87 (16)	94 (24)	0.096
Cholesterol (mg/dL)	167 (43)	178 (41)	0.20
Triglyceride (mg/dL)	121 (51)	139 (94)	0.24
Insulin (mg/dL)	14.9 (11.9)	21.4 (17.4)	0.049
HOMA-IR	3.13 (2.58)	4.71 (4.11)	0.036
C-peptide (μU/mL)	1.57 (1.09)	1.70 (1.03)	0.58
Serum iron (μg/dL)	164 (87)	150 (78)	0.51
Ferritin (mU/mL)	174 (142)	146 (137)	0.38
TNF-α (pg/mL)	31.1 (30.7)	25.8 (29.6)	0.440

Data as mean (SD)

Virological and biochemical findings

Among CHC patients, HCV genotype was 1a in 31 (62%), 1b in 6 (12%), 1a/b in 1 (2%), IIa in 3 (6%) and IIIa in 8 (15%) patients; it was not typeable in 4 patients, and was not tested in 7 patients. HBeAg was positive in 9 (22.5%) CHB patients. HBV DNA load was high (>100,000 copies/mL) in 20 (50%), low (<100,000 copies/mL) in 13 (32.5%), and undetectable in 7 (17.5%) CHB patients.

CHC patients had lower fasting serum insulin and HOMA-IR (Table 1). Fourteen (23.3%) patients with CHC and 9 (22.5%) with CHB had low fasting serum insulin; 13 (21.7%) with CHC and 9 (22.7%) with CHB had HOMA-IR. Other laboratory parameters were similar in patients with CHC and CHB.

Using a linear regression model that included alcohol consumption, CHC was associated with HOMA-IR, indicating that this association was not related to alcohol consumption (<15 g/d).

Histological findings

Liver biopsy was done in 43 (72%) patients with CHC and 20 (50%) with CHB. Mean HAI score, necro-inflammatory grade, fibrosis stage, liver iron score and steatosis score were similar in the two groups (Table 2). Steatosis was seen in 18 (51%) patients with CHC and 10 (59%) with CHB (p=ns).

HOMA-IR showed significant association with stage of fibrosis (p=0.015) in the CHC group but not in the CHB group.

Discussion

In our study, HOMA-IR had significant association

Table 2: Histologic findings in patients with chronic hepatitis C (CHC) and chronic hepatitis B (CHB)

Parameter	HCV (n=43)	HBV (n=20)	*p*
Steatosis score	0.9 (1.2)	1.2 (1.3)	0.382
Iron-staining score	0.6 (1.0)	0.5 (0.7)	0.754
Modified HAI score	10.3 (5.8)	8.9 (6.0)	0.436
Grade	8.5 (4.5)	7.3 (4.9)	0.394
Stage	1.8 (1.8)	1.6 (1.9)	0.760

with stage of fibrosis (p=0.015) in the CHC group but not in the CHB group. This suggests a possible role of insulin resistance in progression of fibrosis.

Custro *et al*[5] reported that the incidence of diabetes mellitus in adults with CHC and CHB (25% and 22.5%, respectively) is four times higher than that in the general population. Patients with chronic hepatitis have impaired glucose metabolism with hyperinsulinemia and insulin resistance. This hyperinsulinemia has been shown to be due to decreased insulin catabolism rather than increased pancreatic insulin secretion.[6,7] Up to 60%-80% of patients with cirrhosis have glucose intolerance, and about 20% eventually develop frank diabetes mellitus.[8] A marked IR is common in patients with liver disease and represents a causative factor for the impaired glucose metabolism seen in these patients.[9] Considering the C-peptide and insulin levels in the present study, hyperinsulinemia both in the CHB and CHC groups was not due to insulin hypersecretion.[10]

Epidemiologic and experimental data suggest that hepatitis C can predispose to nonalcoholic fatty liver disease (NAFLD).[11,12] HCV infection may contribute to the metabolic abnormalities leading to NAFLD and nonalcoholic steatohepatitis (NASH). There is also a theoretical concern that fat in hepatocytes could facilitate HCV infection.[13] In our study, mean HOMA-IR was high both in CHC and CHB patients, but stage of fibrosis was associated with HOMA-IR only in CHC patients. Thus, measurement of fasting serum insulin and HOMA-IR may be recommended in CHC patients as a surrogate marker for evaluating stage of fibrosis. Hui *et al*[1] showed increased HOMA-IR as a predictor of the stage of fibrosis in CHC patients. However, they acknowledged the possibility that the factors responsible for IR may be responsible for progressive fibrosis.

Moreover, BMI and alcohol consumption were associated with grade of hepatitis in the CHC group. Thus, besides detecting predisposing factors for insulin resistance, weight loss and abstinence from alcohol may prevent progression of stage and grade.

In Australia, NAFLD was found in 61% of 148 HCV-infected patients as compared with only 20% in non-HCV-infected adults. Moreover, degree of fibrosis in these patients correlated with the presence of NAFLD, independent of age and obesity.[14] Patients with NAFLD tended to have increased perisinusoidal fibrosis and stellate cell activation, findings that are characteristic of NASH.[15-18]

The increased prevalence of diabetes in HCV has been shown to be predominant among genotype 1- and 2-infected subjects.[19] It is of interest that, despite lower IR levels, subjects with HCV genotype 3 have more extensive hepatic steatosis.[1] The most common HCV genotype in Iran is genotype 1a.[21] In our study, the most frequent genotype was 1a, too. In a retrospective analysis[20] of 1,117 patients with chronic viral hepatitis, logistic regression analysis confirmed that age and HCV infection were independent predictors for diabetes mellitus.

We believe that any study regarding associations between genotype, HOMA-IR and steatosis needs adjustment for other confounders. Some studies suggest that insulin resistance can directly promote fibrogenesis,[21] possibly through the expression of TNF-α level. Because of small sample size, correlation of pretreatment HCV RNA load, HCV genotype and HBV DNA load with IR was not evaluated. We did not consider lifestyle scores, nutrition habits and hours of physical activity of patients.

We recommend that the effect of insulin resistance on CHB and CHC patients be evaluated in a larger sample size and be measured after antiviral therapy and treatment of components of metabolic syndrome in prospective studies.

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