Original Article

Insulin tolerance test is comparable to homeostasis model assessment for insulin resistance in patients with nonalcoholic fatty liver disease

Ajay Duseja, Kiran K Thumburu, Ashim Das,* R K Dhiman, Y K Chawla, S Bhadada, Anil Bhansali****

Departments of Hepatology, *Histopathology and **Endocrinology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012

Introduction: Insulin resistance (IR) is common in patients with nonalcoholic fatty liver disease (NAFLD). We compared the performance of insulin tolerance test and the homeostasis model assessment (HOMA) for measuring IR in such patients. **Methods:** In a prospective study, IR was determined using both insulin tolerance test and HOMA in 22 patients with NAFLD. Rate constant for insulin tolerance test (KITT) was calculated using the formula KITT (%/min) = 0.693/ t½, where t½ was calculated from the slope of plasma glucose concentration during 3-15 minutes after administration of intravenous insulin. IR was assessed using HOMA as the product of fasting insulin (μU/L) and fasting plasma glucose (mmol/L) levels divided by 22.5. **Results:** All the 22 patients had IR. Results of KITT and HOMA–IR for determining IR showed a fair correlation (r = 0.55; p = 0.03). **Conclusions:** Insulin tolerance test may be a useful method for assessing IR in patients with NAFLD. [*Indian J Gastroenterol* 2007;26:170-173]

Nonalcoholic fatty liver disease (NAFLD) has a wide spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH); the latter is believed to predispose to cirrhosis and hepatocellular carcinoma.[1] Insulin resistance (IR) plays a key role in the pathogenesis of NAFLD.[2,3]

Several methods used to assess IR include euglycemic hyperinsulinemic clamp studies, frequently-sampled intravenous (IV) glucose tolerance test (FSIGT), homeostasis model assessment for IR (HOMA–IR), and insulin tolerance test (ITT).[4] Clamp studies, though ideal, are costly and may be cumbersome. Similarly mathematical models like HOMA–IR, which require determination of serum insulin and glucose, may also be costly for routine use. ITT is a simple, quick, reproducible and cheap method of assessing insulin sensitivity and has been validated against clamp studies.[5] However there are no data comparing ITT with HOMA–IR to determine the presence of IR in patients with NAFLD. The present study was aimed at comparing the results of ITT and HOMA–IR for measuring IR in patients with NAFLD.

Methods

Subjects

This prospective study (April 2001 to July 2005) included 22 patients with biopsy-proven NAFLD. All subjects gave an informed consent and the study was approved by our institution's ethical committee. Inclusion criteria were: age >16 years, alcohol intake less than 20 g/day, elevated serum ALT for 6 months (>1.5 fold the upper limit of normal on at least 2 occasions), ultrasound showing hyperechoic liver or other features of steatosis, negative tests for HBsAg, anti-HCV antibody, anti-nuclear antibody, anti-smooth muscle antibody, anti-liver-kidney microsomal antibody, and anti-mitochondrial antibody, normal serum ceruloplasmin, absence of Kayser Fleisher rings on slit lamp examination, normal iron parameters and a liver biopsy consistent with NAFLD.

All patients underwent a glucose tolerance test to look for diabetes mellitus, which was defined as per the WHO criteria, i.e. fasting plasma glucose (FPG) ³126 mg/dL and/or 2-hour plasma glucose ³ 200 mg/dL.[6] Patients with diabetes mellitus and pregnant women were excluded. In addition, 15 age- and gender-matched healthy controls with normal BMI, waist circumference, ALT, FPG and abdominal ultrasound findings were studied as controls.

All patients underwent a detailed physical examination including anthropometry, and were classified as being overweight [body mass index (BMI) ³ 23 but < 25 Kg/m^{2}] or obese (BMI ³ 25 Kg/m^{2}), and as having abnormal waist circumference [> 90 cm (men), >80 cm (women)], as defined by the Asia-Pacific criteria.[7,8] Metabolic syndrome was defined by the presence of three or more modified adult treatment panel III criteria

out of five criteria (abnormal waist circumference, high fasting plasma glucose, high blood pressure, high serum TG, and low serum HDL); abnormal waist circumference was defined as per the Asia-Pacific criteria.[7,9] Fasting serum insulin was measured by radioimmunoassay (Beckman Coulter, Prague, Czech Republic**)** and serum C-peptide by ELISA kits (DRG, Marburg, Germany) in all patients and controls.

IR was determined using ITT and HOMA–IR. Details of insulin tolerance test (ITT) have been reported previously.[3,10] Briefly, after an overnight fast, blood samples were taken for measurement of plasma glucose were collected 15 and 5 minutes before, at the time of, and 3, 6, 9, 12, 15, 20 and 30 minutes after intravenous injection of regular insulin (0.1 U/Kg). All patients received 100 ml of 25% dextrose intravenously at 30 min, irrespective of development of symptoms of hypoglycemia. Rate constant for ITT (KITT) was calculated using formula KITT (%/min) = 0.693/ t½, where t½ was calculated from the slope of the plasma glucose concentration during the period from 3 to 15 minutes after insulin injection, using the least square analysis; the decline in plasma glucose concentration during this period was linear. HOMA-IR was calculated as the product of fasting insulin (μU/L) and fasting plasma glucose (mmol/L) divided by 22.5. Values above 1.64 were taken as abnormal.[2]

Histopathology

Histologically patients were classified into four classes as per Matteoni *et al* (Class 1 = simple steatosis; Class 2 = steatosis + lobular inflammation; Class 3 = + ballooned hepatocytes; Class 4 = + Mallory hyaline or fibrosis); those patients with class 3 or 4 were defined as having NASH.[11] Further, patients with NASH were graded and staged according to Brunt *et al*.[12]

Statistical analysis

Mann-Whitney U test was used to compare values between NAFLD patients and healthy controls. Spearman's correlation was used to assess the relationship between ITT and HOMA–IR**.** P values <0.05 were taken as significant.

Results

Patients

The anthropometric, clinical and biochemical and histological parameters of the 22 patients with NAFLD studied are shown in the Table. The median (range) age of 15 control subjects (10 men) was 28 (24-54) years.

Table: Anthropometric and biochemical data in 22 patients with NAFLD

Parameter	Value
Male: Female	15:7
Age (years)	37 (16-58)
Body mass index (Kg/m$^{2)}$	25.9 (19-36)
Waist circumference (cm)	96 (72-115)
Alanine aminotransferase (IU/L)	106 (53-197)
Insulin (pmol/L)	194.4 (71.5- 1111.2)
Plasma C-peptide (pmol/L)	800 (100 - 2100)
Cholesterol (mg/dL)	204 (124-259)
High density lipoprotein (mg/dL)	37 (25-57)
Low density lipoprotein (mg/dL)	119 (37-198)
Triglycerides (mg/dL)	287 (79- 430)
Hypertension	2 (9%)
Metabolic syndrome	12 (55%)
Histopathology	
Class I	1 (4.5%)
Class II	9 (41%)
Class III	4 (18.2%)
Class IV	8 (36.3%)
NASH (class III+ IV)	12 (55%)
Grade	
1	5 (42%)
2	7 (58%)
3	0
Stage	
0	3 (25%)
1	5 (41%)
2	2 (17%)
3	2 (17%)
4	0

Data are shown either as median (range) or as number (%)

In comparison to healthy controls, all patients with NAFLD showed evidence of IR using both the methods. Patients with NAFLD had significantly lower median KITT (1.86 %/min [range = 0.69-4.54]) than healthy controls (4.31 [1.92-5.93]; p<0.01]. Also, they had higher median HOMA-IR values (8.55 [3-37.4]) than the healthy controls (1.55 [0.69-1.82]; p<0.01).

There was a good correlation between KITT and HOMA-IR values (r=0.55; p=0.03) (Fig).

Discussion

IR is very common in patients with NAFLD.[3,10,13,14] This is believed to be responsible for the 'first hit' in NAFLD, leading to increased lipolysis and hepatic steatosis.[15] Increased fatty acid oxidation, oxidative stress and cytokines may then lead to steatohepatitis in some of these patients. All our patients with NAFLD had evidence of IR using

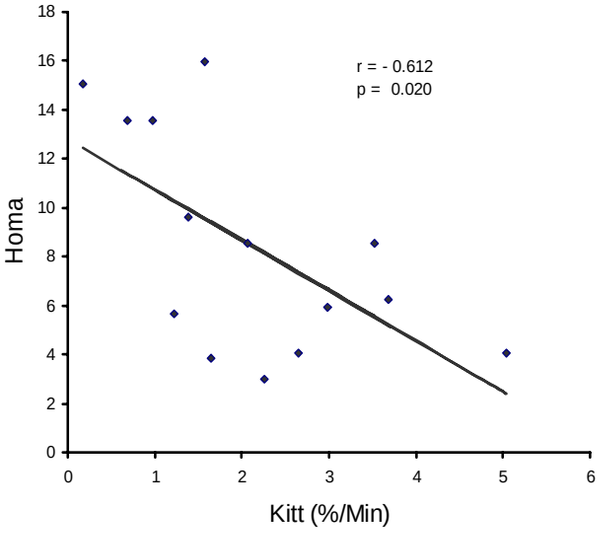

Fig: Graph shows good correlation between rate constant for insulin tolerance test (KITT) and homeostasis model assessment for insulin resistance (HOMA) in determining insulin resistance in patients with NAFLD (r = 0.55; p = 0.03)

both the methods. High (>1) C-peptide to insulin ratio in our patients with NAFLD (Table) is indicative of primary IR in these patients rather than the hyperinsulinemia occurring due to decreased hepatic extraction of insulin due to liver disease (ratio is <1).[2]

Of the several methods available for the detection of IR,[4] clamp studies are cumbersome and costly. HOMA, though easy to do, needs measurement of fasting serum insulin. Further, one needs to simultaneously measure serum C-peptide levels to exclude hyperinsulinemia secondary to liver disease.[2] These two assays make HOMA-IR quite expensive for routine use. In comparison, ITT is a quick and cheap method of assessing IR.

Use of ITT for detection of IR has the limitation that insulin injection induces secretion of counter-regulatory hormones like glucagon, catecholamines, growth hormone and cortisol, which may slow down the uptake of glucose from plasma. However, this response appears at least 15-20 minutes after insulin injection, after the ITT measurement has already been done.[5,16] Thus, the counter-regulatory response is unlikely to influence the measurement of ITT.

Our data showing a fair correlation between the results of ITT and HOMA-IR suggest that ITT may be a useful screening method to look for IR in patients with NAFLD. However, our study had a small sample size. Further, the correlation between ITT and HOMA was not very high (r= 0.55). Hence, there is possibly a need for larger studies on this subject.

In conclusion, we found a fair correlation between the results of ITT and HOMA-IR in patients with NAFLD. It may be possible to use ITT as a screening test for IR in this group of patients.

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Correspondence to: Dr. Duseja, Department of Hepatology. Fax: 91 (172) 274 4401. E-mail: ajayduseja@yahoo.co.in

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Image

Cruveilheir-Baumgarten syndrome revisited

A 60-year-old woman with hepatitis C-related cirrhosis and portal hypertension was admitted with altered sensorium and constipation. On examination, she had encephalopathy, mild pallor and icterus, telengiactasias on the face, and palmar erythema.

Abdominal wall revealed extensive, grossly dilated and tortuous veins all over abdomen and lower chest forming a caput medusae (Fig a). There was also a large coil of veins forming a lump near the supraumbilical area; a palpable thrill with a venous hum was heard over it. The direction of flow in the veins was away from umbilicus.

Ultrasound abdomen revealed multiple anechoic channels on the anterior abdominal wall with large dilated tortuous anechoic channels going towards left branch of portal vein. Spiral CECT in venous phase revealed lobulated liver with irregular margins and altered density. Hepatic veins were distorted. Spleen was enlarged with minimal ascites. There was a coiled up bunch of veins forming a mass like structure on anterior abdominal wall with multiple collaterals adjacent to it, which were connected to left branch of portal vein by a large tortuous patent umbilical vein (Fig b).

Cruveilhier-Baumgarten syndrome is characterized by prominent paraumbilical veins with evidence of portal hypertension. The recanalization of umbilical vein is due to intrahepatic blockade due to cirrhosis of liver. The portosystemic venous collaterals, represented by the umbilical vein, appear on CT as rounded or tubular soft tissue opacities arising in the fatty falciform ligament between the medial and lateral segments of the left lobe of liver. Typically the umbilical vein courses caudally towards the anterior abdominal wall and terminates in subcutaneous collateral veins giving "caput medusae" appearance.[1]

The finding of recanalized umbilical vein and collaterals is often incidental, however these may represent potential sites for hemorrhage, especially during paracentesis or abdominal surgery.[2]

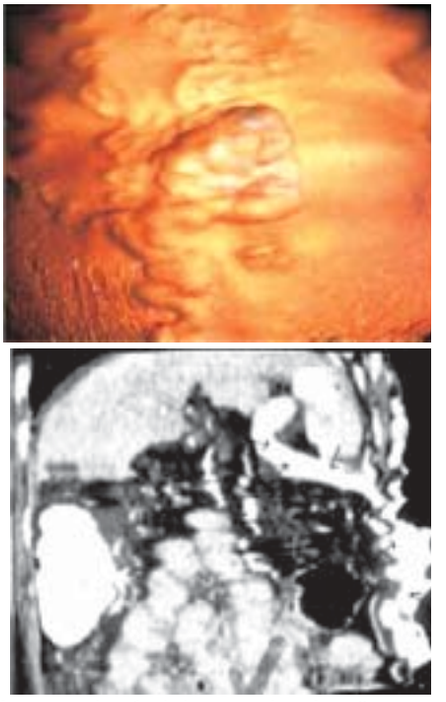

Fig: (a) Photograph of abdomen showing "caput medusa". (b) CT scan (saggital section) showing patent umbilical vein, connecting left branch of portal vein

Jaswinder Singh Sodhi, Showkat Ali Zarger, Mushtaq Ahmad Khan, Gul Javid, Bashir Ahmad Khan, Altaf Hussain Shah, Ghulam Mohd. Gulzar, Manjeet Singh*

Departments of Gastroenterology and *Radiology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Kashmir 190 011

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Correspondence to: Dr MA Khan, Assistant Professor. E-mail: drmushtaqkhan@hotmail.com