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Original Article

Gamma glutamyl transferase is an independent determinant for the association of insulin resistance with nonalcoholic fatty liver disease in Bangladeshi adults
Association of GGT and HOMA-IR with NAFLD

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ABSTRACT

Aims: Nonalcoholic fatty liver disease (NAFLD) is a major cause of liver-related morbidity and is frequently associated with insulin resistance (HOMA-IR) syndrome. Recently serum gamma glutamyl transferase (GGT) has been considered as surrogate marker of NAFLD leading to oxidative stress and hepatocellular damage. In the present study we examined the association of serum GGT and HOMA-IR with NAFLD in Bangladeshi adult subjects.

Materials and methods: Under a cross-sectional analytical design a total of 110 subjects were recruited who came for their routine health check up in the BIHS Hospital, Darussalam, Dhaka, Bangladesh. After whole abdomen ultrasonography, 62 were diagnosed as non-NAFLD and 48 were NAFLD subjects. Serum glucose was measured by glucose-oxidase method, lipid profile and liver enzymes by enzymatic colorimetric method, glycosylated hemoglobin (HbA_{1c}) was measured by high performance liquid chromatography (HPLC), serum insulin were measured by enzyme-linked immunosorbent assay. HOMA-IR was calculated by homeostasis model assessment (HOMA).

Results: NAFLD subjects had significantly higher levels of GGT and HOMA-IR as compared to their non-NAFLD counterparts. Multiple linear regression analysis showed a significant positive association of HOMA-IR with GGT after adjusting the effects of waist circumference (WC) and $HbA_{1c.}$ In binary logistic regression analysis, HOMA-IR and GGT were found to be significant determinants of NAFLD after adjusting the effects of WC and $HbA_{1c.}$

Conclusion: These results suggest that elevated levels of GGT and insulin resistance are more likely to develop NAFLD and thus support a role of these determinants in the pathogenesis of NAFLD in Bangladeshi adult subjects.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of clinical–pathological features ranging from simple steatosis, which is characterized by excessive fat deposition only leading to progression of nonalcoholic steatohepatitis (NASH), which is characterized by oxidative stress, inflammation and hepatocellular damage with or without fibrosis and cirrhosis. NAFLD affects 10–24% of the general population from different countries and recognized increasingly as a major cause of liver related morbidity and mortality [1,2]. NAFLD is a consequence of insulin resistance-related diseases, such as obesity, metabolic syndrome, atherosclerosis, and type 2 diabetes [3].

The pathogenesis of NAFLD appears to involve a multi-hit process. The first hit is the steatosis which is believed to be triggered by insulin resistance (IR), and the second hit, which involves cytokines alteration and oxidative stress, results in

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disease progression. Several investigators have reported that NAFLD is significantly associated with reduced insulin sensitivity due to impaired insulin signaling [4]. Free fatty acid (FFA) released form dietary fat can be esterified with glycerol in to triglycerides (TG) which then deposited in the adipocytes. Excessive accumulation of liver fat reduces the insulin stimulated glucose uptake and develops insulin resistant related metabolic abnormalities [4,5].

By hyperinsulinaemic-euglycaemic clamp technique, it has been shown that IR is a pathophysiological determinant of independent of obesity and abdominal adiposity. Insulin resistant subjects with NAFLD show reduced insulin sensitivity not only at the level of the muscle but also at the level of the liver and adipose tissue [6]. Several clinical and epidemiological studies revealed that NAFLD is more closely associated with IR rather than metabolic syndrome itself and associates with its clinical manifestations of metabolic impairments [7,8].

Subjects when develop NAFLD do not have symptoms or signs of liver related diseases but have abnormalities in liver function enzymes (usually 1–2 times upper limit of the reference). It is clinically diagnosed by transaminitis with an asymptomatic elevation of aminotransferases in 42–90% [9] and fatty liver changes on ultrasound. Excessive alcohol consumption induces elevation of serum aminotransferase levels in the western population is a common cause of NAFLD and also an alarming problem in the Asia-Pacific region [10]. Mild to moderate elevation of serum aminotransferases is the most common and often the only laboratory abnormality found in patients with NAFLD and also considered as early surrogate markers of NAFLD [11]. Studies also indicated that elevated activities of these enzymes are associated with metabolic syndrome and precedes the clinical manifestations of other metabolic derangements [11,12].

Several studies indicated that alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) are independent predictors of NAFLD [11,13]. An increased level of ALT – a glucogenic enzyme synthesized in liver demonstrates to be an indicator of impaired insulin signaling and develops hepatic IR [14]. On the other hand, serum GGT – a hepatobiliary enzyme synthesized in epithelial cells of the intrahepatic duct and closely related to hepatic steatosis and considered as a surrogate marker of NAFLD [4]. The underlying mechanisms whereby elevated GGT responsible for the development of hepatic steatosis have not been clearly defined. One possible explanation by Ortega et al. proposed that increased deposition of liver fat induces hepatocellular damage and simulate the synthesis of GGT [15]. These increased levels of GGT enhance mitochondrial damage, and free radicals that cause significant oxidative stress and proinflammation. Since GGT is a cell surface protein involved in cellular glutathione (GSH) metabolism which is the main intracellular thiol antioxidant agent in mammalian cells. Elevated levels of GGT induces GSH hydrolysis to produce cyseinyl-glyceine, which then oxidized to generate reactive oxygen species (ROS) such as hydrogen peroxidie (H_2O_2), superoxidide (O_2^-) and hydroxyl (OH^-) anion radicals through its interaction with free iron and induces low grade hepatic inflammation by hepatic steatosis. A number of recent studies have proposed that GGT may be a simple and reliable marker of visceral and hepatic fat deposition and hepatic steatosis which can lead to hepatic IR and long term hepatic IR may lead to metabolic abnormalities [16].

In previous studies, the relationships of liver enzymes particularly ALT, AST and their ratio with IR in NAFLD subjects has been clearly evaluated. However, studies with GGT in association with IR in NAFLD subjects are rare or very limited study has been studied. The aim of this study is to evaluate whether an independent relationship exists between IR and serum GGT levels with NAFLD in Bangladeshi adult subjects.

2. Materials and methods

2.1. Study design and subjects

We performed a cross-sectional analytical study with group comparison design and a total number of 110 (one hundred and ten) prediabetic subjects were purposively recruited in the study irrespective of race, religion and socioeconomic status. Diabetes and prediabetes were diagnosed following WHO Group Study criteria [17] who were attending the Bangladesh Institute of Health Sciences (BIHS) Hospital, Darussalam, Dhaka, Bangladesh. Of the total, upper abdomen ultrasonogram had done and the subjects were divided into 62 without NAFLD and 48 NAFLD groups. Subjects suffering from any systemic illness like acute severe septic conditions, acute and chronic cardiac disease, hepatic, renal, acute and chronic respiratory failure, history of alcohol addiction, drugs affecting liver enzymes, hepatitis C virus, hepatitis B virus surface antigen, SGOT and GGT levels more than three time the normal, suffering from cancer, stroke, type 1 diabetes, recent change ($\geq$10%) in body weight, current medication and pregnant subjects was excluded from the study. After taking brief history, preliminary selection was done, and the purpose of the study was explained in details to each subject and their verbal consent was taken. After overnight (8–14 h) fasting subjects reported at morning in the hospital between 8.00 and 9.00 am and informed written consent was taken from them. Venous blood ($\sim$6 ml) from each participant was obtained by venipuncture following standard procedure and serum separated from the blood used for biochemical measurements. A predesigned case record form was used to record relevant clinical, medical, demographic, socio-economic data such as age, sex, educational status and occupational status from the consenting subjects. Anthropometric indices including waist and hip circumference (WC & HC), weight, height, systolic and diastolic blood pressures (SBP and DBP) were measured by standard clinical procedures on the very first day of the visit. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The study was approved from the Ethical Review Committee of Bangladesh Diabetic Association (BADAS). Ref no: BADAS-ERC/13/00106. Each participant gave written informed consent prior to study inclusion.

2.2. Radiological examinations

Abdominal ultrasounds were performed by a well trained physician using a 3.5 MHz linear transducer (Philips Ultrasound-Ay-MNT-15 TTK, HDI-4000, Netherlands) in fasting state for grading the extent of fatty liver and to look for evidence of portal hypertension. NAFLD was defined as any degree of fatty liver in the absence of alcohol intake. NAFLD, if present, was classified based on standard ultrasonographic criteria as: Grade 0: normal echogenicity; Grade 1 (mild steatosis): slightly increased liver echogenicity with normal vessels and absent posterior attenuation; Grade 2 (moderate steatosis): moderately increased liver echogenicity with partial dimming of vessels and early posterior attenuation; Grade 3 (severe steatosis): diffusely increased liver echogenicity with absence of visible vessels and heavy posterior attenuation [18].

2.3. Biochemical analysis

Serum glucose was measured by glucose-oxidase method, serum lipid profile [total cholesterol (TC), triglyceride (TG), and high density lipoprotein cholesterol (HDL-c)], clinical liver enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) were