Role of γ-glutamyl transferase levels in prediction of high cardiovascular risk among patients with non-alcoholic fatty liver disease

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Background & objectives: Non-alcoholic fatty liver disease (NAFLD) is an important cause of elevated liver functions. There is evidence showing an association between NAFLD and subclinical atherosclerosis independent of traditional risk factors. We undertook this retrospective study to determine the association of Framingham cardiovascular risk scoring system with liver function tests and inflammatory markers and to find the role of liver function tests in determination of CVD risk among non-obese and non-diabetic subjects with non-alcoholic fatty liver disease.

Methods: A total of 2058 patients were included in the study. Framingham cardiovascular risk scoring was done of all patients according to the age, gender, systolic blood pressure, serum total cholesterol and HDL cholesterol levels, smoking and antihypertensive medication history. Liver function test, lipid profile, insulin, uric acid, ferritin levels, *etc*. were determined.

Results: According to the ultrasonography findings, patients were grouped as without any fatty infiltration of the liver (control group) (n=982), mild (n= 473), moderate (n=363) and severe fatty liver disease (n= 240) groups. In severe fatty liver disease group, the mean Framingham cardiovascular risk score was significantly higher than that of other groups. There was a positive correlation between GGT, uric acid and ferritin levels with Framingham cardiovascular score. In multivariate analysis, high GGT levels were positively associated with high-risk disease presence (OR: 3.02, 95% CI: 2.62-3.42) compared to low GGT levels independent of the age and sex.

Interpretation & conclusions: Cardiovascular disease risk increases with the presence and stage of fatty liver disease. Our findings showed a positive correlation between elevated GGT levels and Framingham cardiovascular risk scoring system among non-diabetic, non-obese adults which could be important in clinical practice. Though in normal limits, elevated GGT levels among patients with fatty liver disease should be regarded as a sign of increased cardiovascular disease risk. Larger studies are warranted to elucidate the role of GGT in prediction of cardiovascular risk.

Key words BMI - cardiovascular disease risk score - Framingham - gamma glutamyl transferase - non-alcoholic fatty liver disease - non-obese

Non-alcoholic fatty liver disease (NAFLD) is characterized by morphological features observed in alcohol-related liver disease in patients without significant alcohol consumption[1]. It has been shown that NAFLD is responsible approximately for 90 per cent of distorted liver function tests (LFTs) in patients without any known liver disease[2]. Insulin resistance, oxidative stress and inflammatory period are thought to be responsible in not only initiation but also progression of fatty liver disease. Nowadays, non-alcoholic fatty liver disease gains more importance with its increasing prevalence, potential to develop liver failure and cirrhosis, and morbidities with concomitant diseases[1].

Obesity (body mass index, BMI>30kg/m^2) and type 2 diabetes mellitus are defined risk factors for fatty liver disease development[3]. However, in clinical practice, NAFLD is also observed among subjects without any known risk factors. In a study determining the independent predictors of lean (non-obese) patients with NAFLD; younger age, female sex, and a decreased likelihood of having insulin resistance (IR) and hypercholesterolaemia were found as independently associated with lean NAFLD[4]. Since NAFLD is regarded as the hepatic manifestation of metabolic syndrome, it has been shown as a threat in cardiovascular disease (CVD) development after exclusion of classical risk factors[5]. The prevalence and incidence of CVD have been found to be increased in individuals with NAFLD[6,7]. Moreover, in a systematic review of 27 studies, it is concluded that there is evidence to support the association of NAFLD with subclinical atherosclerosis independent of traditional risk factors and metabolic syndrome[8].

The Framingham risk score (FRS) that was first developed based on data obtained from the Framingham Heart Study, is a gender-specific algorithm used to estimate the 10-year cardiovascular risk of an individual[9]. Framingham risk score is useful in determination of individuals who benefit from lifestyle modification and preventive medical treatment.

The aim of this retrospective study was to determine the association of Framingham cardiovascular risk scoring system with liver function tests and inflammatory markers and to find the role of liver function tests in determination of CVD risk among non-obese and non-diabetic patients with non-alcoholic fatty liver disease.

Material & Methods

This retrospective study was carried out in Turgut Ozal University Hospital, Ankara, Turkey, on all non-obese (BMI <30 kg/m^2) and non-diabetic 2058 patients (1563 female and 495 male) admitted to the Internal Medicine department with any complaint other than cirrhosis, jaundice, or chest pain between March 2009 and May 2010. Data were obtained from patient records. Exclusion criteria were as follows: a significant history of alcohol use (>30 g for male, >20 g for female), body mass index > 30 kg/m^2, positive results for HBsAg or anti-HCV, autoimmune hepatitis, Wilson's disease, haemochromatosis, any known chronic liver disease, malignancies, diabetes mellitus, thyroid disease, atherosclerotic heart disease or renal disease. The study protocol was approved by the university ethics committee.

Height and weight of all participants were measured and the BMI was calculated. Diabetes was defined as a fasting plasma glucose (FPG) level greater than 125 mg/dl or a self-reported diagnosis of diabetes and use of specific therapy. Impaired fasting glucose (IFG) was diagnosed if FPG was 110-125 mg/dl and 2-h post-load glucose (75 g) was <140 mg/dl and the individual was not a known diabetic[10]. Subjects with BMI > 30 kg/m^2 and diagnosis of diabetes mellitus were excluded from the study. Waist circumference (cm) and blood pressures of all patients were recorded.

Laboratory tests: Liver function tests [including alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and bilirubin levels], lipid profiles [including total cholesterol, triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein levels (LDL)], C-reactive protein (CRP), fasting blood glucose (FBG), insulin, thyroid stimulating hormone (TSH), uric acid and ferritin levels were studied in all patients after an overnight fast using the standard methods. Insulin resistance was measured using the homeostatic model of the assessment of insulin resistance (HOMA-IR) and was obtained by applying the following formula[11]:

HOMA: fasting insulin (IU/ml) × fasting blood glucose (mmol/l)/22.5

Framingham cardiovascular risk scoring system: The cardiovascular risks of all subjects were determined using the Framingham risk scoring system according to the age, gender, systolic blood pressure, serum total and HDL cholesterol levels, smoking and hypertension treatment histories of subjects[12]. According to the Framingham cardiovascular disease risk score; the

10-yr risk of CVD was classified as low (<10%), moderate (10 to 20%), or high (>20%)[13].

Fatty liver disease diagnosis and evaluation: All subjects underwent a liver ultrasonography scanning and the presence and stages of fatty liver were graded. Liver steatosis was scored on a scale of 0-3; 0, absent; 1, mild; 2, moderate; 3, severe. Steatosis was graded on the basis of abnormally intense, high level echoes arising from the hepatic parenchyma, liver-kidney difference in echo amplitude, echo penetration into deep portion of the liver and clarity of liver blood vessel structure[14].

Statistical analysis: All analyses were performed with the Statistical Package for Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA) for Windows 17.0 program. Comparisons of demographic features of groups were performed with Fisher's exact test and Pearson χ^2 tests. Significant differences were determined between the groups with ANOVA test. Logistic regression model was used to determine the independent predictors of high cardiovascular risk (CVR) patients, while comparisons between groups were made using two-tailed Student's t test.

Results

Two thousand and fifty eight non-obese, non-diabetic inpatients (1563 women, 495 men) with the mean age of 49.7±13.2 yr (ranging between 21-70 yr) were included in the study. They were grouped according to their ultrasound findings as follows; 982 (47.7%) cases without any fat accumulation in liver were regarded as control group; and among the remaining, 473 (22.9%) had mild, 363 (17.6%) had moderate and 240 (11.6%) had severe fatty liver disease. The general characteristics and laboratory findings of the groups are shown in Table I.

When grouped according to the presence and severity of fatty liver disease, there was no significant difference between groups with regard to the age. However, when evaluated with regard to gender; there was higher number of men in severe NAFLD group compared to the control group (P<0.01). In evaluation of BMI and waist circumference, with the increasing grade of NAFLD, BMI and waist circumference also increased though all participants of the study were non-obese.

With the increasing grade of NAFLD, the ALT, AST and GGT levels also increased. Similarly, CRP, uric acid and ferritin levels also increased with the increasing stage of NAFLD and the difference between the control group and grade 3 NAFLD groups was significant (P<0.01).

Though patients with diabetes were not included in the study, 185 cases (9.0%) had impaired fasting glucose (IFG). Among these 185 patients, nine were in control group, 17 were in NAFLD stage 1, 69 in NAFLD stage 2 and 90 were in NAFLD stage 3 group. With the increasing stages of fatty liver disease, the presence of IFG also increased significantly (P<0.05). There was a significant increase in risk scores among NAFLD stage 3 group when compared with the control group (P<0.01).

A significant correlation was found between Framingham cardiovascular risk scoring system and GGT, uric acid and ferritin levels in logistic regression analysis (Table II).

When the patients were grouped according to the Framingham cardiovascular disease risk score as high (risk score >20%), moderate (risk score >10, <20%) or low (risk score <10%) risk, 226 were in high CVR group and among them, 56, 49, 64, 51 were in control group, grade 1, grade 2, grade 3 fatty liver disease groups, respectively. When the groups were compared according to the presence of patients with high CVD risk; the difference between all fatty liver disease groups and control group was significant (P<0.01).

When the sample was divided into quartiles of GGT levels, increase in GGT levels was associated with the increase in prevalence of high-risk subjects. The sensitivity and specificity levels of some cut-off points of GGT for the whole group in diagnosis of high-risk subjects are presented in Table III. In ROC curve analysis the optimum cut-off value for GGT in diagnosis of high CVR subjects was 22.5 IU (Figure). In multivariate analysis, high GGT levels were positively associated with high-risk disease presence (OR: 3.02, 95% CI: 2.62-3.42) compared to low GGT levels independent of the age and sex.

Discussion

In this study of 2058 non-diabetic, non-obese patients, fatty liver disease was observed in 52.3 per cent, although two major risk factors, obesity and diabetes mellitus, were not present. Moreover, 11.6 per cent patients had the severe fatty liver disease. The cardiovascular risk, evaluated with Framingham risk scoring system, increased among fatty liver disease patients compared with control group. Another major

Table I. Clinical and biochemical characteristics of study population				
Parameters	Control (n=982)	NAFLD stage 1 (n=473)	NAFLD stage 2 (n=363)	NAFLD stage 3 (n=240)
Age (yr)	49.5 ± 9.7	49.2 ± 11.2	50.1 ± 13.7	51.7 ± 9.3
Gender (female/male) (%)	792/190 (80)	380/93 (80)	273/90 (75)	118/122[δ] (49)
BMI (kg/m²)	25.4 ± 3.2	26.9 ± 2.9	27.6 ± 2.1	28.8 ± 1.2
Waist cicumference (cm)	92.2 ± 2.3	96.3 ± 3.2	98.1 ± 1.3	98.9 ± 1.2
FBG (mg/dl)	89.5 ± 11.2	91.4 ± 9.7	91.4 ± 10.4	98.1 ± 11.4[δ]
ALT (IU/l)	19.7 ± 9.2	20.5 ± 8.7	25.6 ± 9.8	28.2 ± 10.7
AST (IU/l)	18.5 ± 7.6	19.2 ± 9.2	25.3 ± 11.4	30.2 ± 11.1
AST/ALT	0.93 ± 0.12	0.92 ± 0.11	0.94 ± 0.12	0.98 ± 0.9
GGT (IU/l)	21.2 ± 11.2	23.2 ± 10.2	26.4 ± 12.4	36. 2 ± 13.2
ALP (IU/l)	74.2 ± 16.4	77.1 ± 18.2	81.6 ± 19.1	84.7 ± 23.1
Total cholesterol (mg/dl)	198.8 ± 43.2	205.1 ± 52.1	198.2 ± 51.2	203.5 ± 56.2
HDL cholesterol (mg/dl)	59.2 ± 11.1	55.4 ± 9.2	52.9 ± 8.8	48.2 ± 8.7[*]
LDL cholesterol (mg/dl)	118.9 ± 32.1	121.1 ± 38.7	118.2 ± 27.2	116.1 ± 25.1
Triglyceride (mg/dl)	115.6 ± 61.2	134.5 ± 62.1	136.0 ± 54.1	141.1 ± 68.7
CRP (mg/l)	4.2 ± 2.6	4.3 ± 3.1	5.5 ± 4.1	5.4 ± 3.9[*]
Uric acid (mg/dl)	4.4 ± 1.0	4.3 ± 1.0	4.9 ± 1.0	5.2 ± 1.1[*]
Ferritin (ng/ml)	48.6 ± 12.4	57.6 ± 19.8	94.8 ± 32.4	96.2 ± 31.2[*]
HOMA-IR	1.5 ± 1.4	2.6 ± 0.9[*]	3.6 ± 1.1	3.7 ± 1.0[*]
Framingham risk score	2.89 ± 1 .4	2.92 ± 1.5[*]	5.0 ± 2.8	6.4 ± 3.6[*†]

BMI, body mass index; FBG, fasting blood glucose; ALT, alanine amino transferase; AST, aspartate amino transferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; HDL, high density lipoprotein; LDL, low density lipoprotein levels, CRP: C-reactive protein; HOMA-IR, homeostatic model of the assessment of insulin resistance
[*]*P*<0.01 compared with control; [†]*P*<0.01 compared with stage 1; [δ]*P*<0.01 compared with stage 2

finding of this study was the association of higher GGT levels with the increased cardiovascular disease risk. This finding should be confirmed with larger studies and the upper limits of GGT may be discussed according to the new roles of GGT in diagnosis of different diseases.

Similar to our findings, higher Framingham cardiovascular risk score has been reported among patients with fatty liver disease[15]. Increased incidence of coronary artery disease (CAD) has been reported among patients with fatty liver disease[16]. In a study by Acıkel *et al*[17] fatty liver disease has been identified as an independent risk factor for CAD and GGT has been suggested as a predictor for the presence or severity

of CAD. Different mechanisms have been proposed to elucidate the association of fatty liver disease with CAD including insulin resistance, endothelial dysfunction and resulting decrease in nitric oxide levels[18] and decreased levels of adiponectin which may result in both glucose intolerance and increased atherosclerotic processes[19].

We found a significant positive correlation between Framingham cardiovascular risk scoring system and GGT, uric acid and ferritin levels. GGT has been reported to correlate with insulin resistance in a study on fatty liver disease due to varying aetiology[20]. Elevated GGT levels have been reported to be of prognostic significance in coronary artery disease[21].

Table II. Results of logistic regression analysis in prediction of high cardiovascular disease risk

Factor	Beta value	95% Confidence Interval	P value
ALT	1.06	1.00-1.14	0.73
AST	1.06	0.93-1.21	0.95
GGT	0.19	0.13- 0.28	0.01
ALP	0.98	0.97-1.002	0.34
Ferritin	0.17	0.09-0.37	0.04
CRP	0.49	0.31-0.79	0.111
Uric acid	0.46	0.19-1.92	0.001
HOMA-IR	0.065	0.001-0.02	0.38

Abbreviations: same as given in Table I

Table III. Sensitivity and specificity values of gamma glutamyl transferase (GGT) levels in diagnosis of high-risk subjects

| GGT (IU/ml)	Sensitivity	Specificity	LR +	LR -
18.5	0.91	0.51	1.86	0.18
19.5	0.91	0.59	2.21	0.15
20.5	0.90	0.68	2.81	0.14
21.5	0.90	0.74	3.46	0.13
22.5	0.82	0.78	3.72	0.23
23.5	0.78	0.84	4.8	0.26
24.5	0.76	0.86	5.4	0.27

LR, likely hood ratio

In our study, GGT rather than ALT or AST was found to have a positive correlation with Framingham cardiovascular risk scoring system. In a study among 7613 middle-aged men with a 11.5 years follow up period, elevated GGT levels were shown to increase all-cause and cardiovascular mortalities[22]. Similarly, in another population-based large study elevated GGT levels were found to be associated with the increased cardiovascular mortality[23]. In an earlier study we reported a positive association between a high GGT level and cardiovascular disease prevalence[24]. Hepatic steatosis and increased carotid intima-media thickness, a sign of atherosclerosis, have been shown to be associated with normal or slightly elevated GGT levels[25]. In a study conducted on middle-aged African population, GGT levels were reported to be independently associated with insulin sensitivity and metabolic syndrome and chronic elevation of GGT was suggested as an indicator of high risk for the development of these metabolic disorders[26].

The association of increased ferritin levels with fatty liver disease has been studied before. In a recent retrospective analysis of 1014 patients with liver biopsy-confirmed NAFLD, serum level of ferritin was shown to correlate with severe liver fibrosis. However, in the same study serum ferritin levels alone were found to have a low level of diagnostic accuracy for the presence or severity of liver fibrosis in patients with NAFLD[27].

Patients with fatty liver disease tend to have higher serum uric acid values. It has been reported that increased uric acid levels is associated with oxidative stress and insulin resistance[28]. Insulin resistance has been shown not only to increase the synthesis of uric acid but also decrease its excretion[29]. Uric acid is deemed to stimulate vascular smooth muscle proliferation and endothelial dysfunction[30]. Among patients with fatty liver disease, elevated serum uric acid levels may accompany vascular inflammation and arterial damage, increasing the cardiovascular disease risk. In this aspect, it can be suggested that the uric acid lowering treatments may help to diminish cardiovascular disease risk.

In this study we determined the presence of fatty liver disease with ultrasound, not biopsy. However, semi-quantitative ultrasound indices were correlated with the

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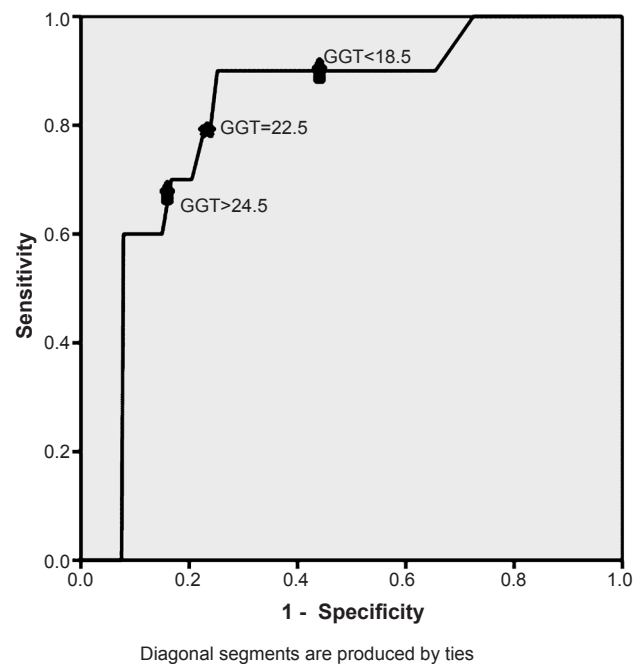
Figure. ROC curve of GGT in prediction of high CVD risk (Area under the curve. 0,862).

metabolic derangements and histological features in the NAFLD spectrum. The main strength of this study was the exclusion of obese and diabetic patients. Our study had some limitations. First, the participants were not a random sample, and, therefore, the results may not be generalized to other populations. Second, this study was retrospective in nature and the follow up information or cardiovascular outcome of the patients were not known. And lastly, FRS has been suggested to overestimate CVD risk in population of non-British descent which may also be true for our population[31].

In conclusion, cardiovascular disease risk increases with the presence and stage of fatty liver disease. A positive correlation between elevated GGT levels and Framingham cardiovascular risk scoring system among non-diabetic, non-obese adults is an important observation of this study. Though in normal limits, elevated GGT levels among patients with fatty liver disease should be regarded as a sign of increased cardiovascular disease risk. Larger studies are warranted to elucidate the role of GGT in prediction of cardiovascular risk.

Acknowledgment

Conflicts of Interest: None.

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