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Non-alcoholic Fatty Liver, Risk Factors and Diagnostic Challenges in the Georgian Population

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease in human history, characterized by the excessive accumulation of lipid granules in liver hepatocytes (steatosis) in the absence of other etiologies, such as excessive alcohol intake or the use of hepatotoxic drugs. Multiple risk factors predispose to the development and progression of NAFLD. Developing reliable and practical tools for diagnosing NAFLD during disease screening is crucial for the early detection and effective treatment of NAFLD. Early diagnosis may help apply appropriate preventive measures to prevent the progression of advanced liver diseases. Several clinical indices have been evaluated for the diagnosis of NAFLD, including the Fatty Liver Index (FLI), the Hepatic Steatosis Index (HSI), and the Lipid Accumulation Product (LAP). This non-invasive testing, combined with ultrasound, can be used as a powerful method for diagnosing NAFLD.

Objectives: This study aimed to identify the primary risk factors of NAFLD in an adult population in Georgia and compare clinical indices with hepatic ultrasound data for the diagnosis of NAFLD.

Methods: A total of 360 subjects were included in the study. We evaluated for BMI, waist circumference, presence of hypertension, diabetes, metabolic syndrome, ALT, AST, GGT, serum lipids, albumin, and CBC.

Results: There was no significant difference in age between the groups. There was also no statistically significant difference between the groups in terms of physical activity and the incidence of chronic kidney disease. In patients with NAFLD, the incidence of diabetes, dyslipidemia, metabolic syndrome, and hypertension was significantly higher compared to the control group. As laboratory studies have shown, NADL patients exhibited significantly higher levels of transaminases, triglycerides, glucose, and platelets, as well as significantly lower levels of albumin. In 250 patients with NAFLD, we studied clinical indices and ultrasound grading of this disease. Grade 1 had 88(35.2%) patients; grade 2 – 104(41.6%) and grade 3 – 58(23.2%) patients. FLI incidence in patients with NAFLD with a cat-off value of score >30 and <60 was 222 (88.8%). HSI incidence in patients with NAFLD with a cat-off value of score >36 was 203 (81.2%), and LAP incidence in patients with NAFLD with a cat-off value of score >30 was 188 (75.2%). The sensitivity and specificity of FLI for diagnosing NAFLD were 95.7% and 73.6%, respectively. For HSI, they were 91% and 70%, respectively, and for LAP, they were 93.5% and 86%. The study of clinical indices and comparison with ultrasound data showed that all clinical indices correlate with the grades of hepatic steatosis.

Conclusions: In conclusion, we analyzed the medical examination information of the Georgian population, and the results showed that the occurrence of NAFLD is closely related to METs. Individuals should manage their BMI, blood glucose levels, hypertension, blood lipids, and liver function tests to prevent the development of NAFLD. Our study verifies the accuracy of clinical indices, including FLI, HIS, and LAP, for diagnosing NAFLD as assessed using ultrasound examination. Therefore, clinical indices, combined with ultrasound, are a powerful and valuable diagnostic tool for detecting NAFLD, potentially reducing the need for MRI and liver biopsy.

Keywords: Fatty liver index (FLI); hepatic steatosis index (HSI); lipid accumulation product (LAP); non-alcoholic fatty liver disease (NAFLD); ultrasound.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease in human history, characterized by the excessive accumulation of lipid granules in liver hepatocytes (steatosis) in the absence of other etiologies, such as excessive alcohol intake or the use of hepatotoxic drugs.[1-3] NAFLD is now the most common cause of chronic liver disease in the developed world and is a leading indication for liver transplantation in the United States (US).[4] The global prevalence of NAFLD is estimated to be 25%, with the lowest prevalence in Africa (13.5%) and the highest in the Middle East (31.8%) and South America (30.4%). About 33% of people worldwide have NAFLD, with rates increasing from 25 to 38% in the last thirty years.[5] Multiple risk factors predispose to the development and progression of NAFLD. In most studies, the prevalence is higher in individuals with features of metabolic syndrome (METs), including obesity, diabetes, hypertension, and an increased waist circumference. Furthermore, several studies have suggested that the presence of these factors also increases the likelihood that people have more advanced forms of NAFLD.[6]

Developing reliable and practical tools for diagnosing NAFLD during disease screening is crucial for the early detection and effective treatment of NAFLD. Early diagnosis may help apply appropriate preventive measures to prevent the progression of advanced liver diseases. Patients with advanced liver fibrosis are at an increased risk of liver-related events (gastroesophageal varices, bleeding, ascites, and hepatocellular cancer) and have higher mortality.[7-9] While liver biopsy is considered the gold standard technique for diagnosing hepatic steatosis and fibrosis, it is an invasive procedure. It may result in clinical complications.[10] Liver elastography is a type of elastography procedure that obtains measurements of hepatic fat and fibrosis with high accuracy for identifying early stages of fibrosis compared to advanced stages, relative to liver biopsy.[11] Some non-invasive imaging

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techniques have been proposed as alternative methods to liver biopsy, which include transient elastography, magnetic resonance imaging (MRI), and computed tomography. Abdominal ultrasonography is usually sufficient to detect hepatic steatosis and is the recommended primary diagnostic tool for NAFLD.[12] However, these can be expensive and not readily available.[13] Abdominal ultrasonography is typically sufficient for detecting hepatic steatosis and is the recommended primary diagnostic tool for NAFLD.[14] The underlying mechanism of hepatic steatosis diagnosis through ultrasound is based on increased liver echogenicity due to the accumulation of intracellular lipid droplets. In severe steatosis, visualizing the underlying structures is difficult due to the weak penetration of sonic radiation in the liver.[15,16] Therefore, reproducible and straightforward serum biomarkers and scoring systems are necessary for the diagnosis of hepatic steatosis.

Several clinical indices have been evaluated for the diagnosis of NAFLD:

- The Fatty Liver Index (FLI) represents a non-invasive and well-predictive algorithm for estimating hepatic steatosis. The established method relies on BMI, waist circumference, serum TG, and gamma-glutamyl transferase (GGT) measurements;[17]
- The hepatic steatosis index (HSI) is a screening tool designed to optimize NAFLD and NASH management, helping physicians identify candidates for liver ultrasound and those who require lifestyle modification;[18,19]
- The Lipid Accumulation Product (LAP) is a straightforward algorithm that considers gender, waist circumference, and fasting triglyceride levels.[20,21]

Despite the above, currently available methods, including clinical indices and imaging, cannot replace the need for liver biopsy in diagnosing NAFLD. Therefore, this study aimed to identify the primary risk factors of NAFLD in an adult population in Georgia and compare clinical indices with hepatic ultrasound data for the diagnosis of NAFLD.

METHODS

Study population

We studied 360 subjects who visited the Tbilisi State Medical University and the TSMU and Ingorokva High Medical Technologies University Clinic between 2022 and 2024. The subjects were selected based on the following inclusion criteria: age over 18 years, no pregnancy or breastfeeding, no history of alcohol consumption or alcohol consumption of less than 20 mg per day in women and less than 30 mg per day in men, and no hepatitis C, B, liver cancer, biliary disease, autoimmune diseases, cancer, or hereditary disorders, no history of drug use that could result in liver steatosis, like corticosteroid consumption. For the identification of risk factors of NAFLD, the patients were divided into a NAFLD group (250 subjects) and a non-NAFLD control group, which consisted of 110 subjects.

Methods

Demographic data and the participants' status of alcohol consumption, physical activity, smoking, and medical history, including hypertension, cardiovascular disease, diabetes, gastrointestinal disease, and stroke, were studied.

Weight and height were measured to calculate the body mass index (BMI) in kg/m². Waist circumference was measured midway between the lower costal margin and the iliac crest. After twelve hours of fasting, venous blood samples were drawn for laboratory assessment of serum lipid profiles, liver enzymes: aspartate aminotransferase (AST), alanine transaminase (ALT), Gamma-glutamyl transpeptidase (GGT), and fasting blood sugar, albumin, and CBC. Samples were analyzed in the local laboratory with a standard method.

In patients, we studied the following clinical indices:

- FLI was calculated based on the following formula: ey/(1 + ey) × 100, where: y = 0.953 ×triglycerides (TGs) (mg/dL) + 0.139 × BMI (kg/m2) + 0.718 × GGT(U/L) + 0.053 ×WC (cm) − 15,745. Values of FLI ranged from 0 to 100 and were classified into three categories: <30 – no steatosis; 30-59 -intermediate state, and ≥60 – hepatic steatosis;
- HSI = 8 × (ALT / AST ratio) + BMI +2, if female; +2, if diabetes mellitus. HSI values below 30 indicate that NAFLD can be ruled out (with a negative likelihood ratio of up to 0.186). HSI values of 36 and above indicate that a NAFLD-positive diagnosis is highly likely (with a positive likelihood ratio starting at 6.069);
- LAP for men = (Waist circumference(cm)-65) x triglycerides (mmol/l);
- LAP for women = (Waist circumference(cm)-58) x triglycerides (mmol/l); There are no universally defined normal values for LAP, but studies suggest that in healthy individuals, LAP values tend to be below 30 cm×mmol/l.

All individuals submitted to liver ultrasound to diagnose NAFLD based on the percentage of liver fat. Grading of fatty liver changes was performed as follows:

- Grade 1: Increased liver echogenicity without fading around the portal vein and diaphragm;
- Grade 2: Increased liver echogenicity with fading of the portal vein but without fading of the diaphragm;

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- Grade 3: Increased echogenicity of the liver with the disappearance of the portal vein and diaphragm.

Statistical analysis

Statistical analysis was performed using SPSS 27.0 software. Categorical variables are presented as numbers (%), and continuous variables are expressed as means ± standard deviations (SD), and compared between groups using Student's t-test. Confidence intervals (CIs) for sensitivity and specificity were calculated and reported. A p-value <0.05 was considered significant in the analysis.

RESULTS

The clinical and biochemical characteristics of the 360 patients with NAFLD and the control group are described in Table 1. Among the participants with NAFLD, 186 (74.4%) were males, and 64 (25.6%) were females; 147 (58.8%) were smokers. There was no significant difference in age between the groups. There was also no statistically significant difference between the groups in terms of physical activity and the incidence of chronic kidney disease. In patients with NAFLD, the incidence of diabetes, dyslipidemia, metabolic syndrome, and hypertension was significantly higher compared to the control group.

TABLE 1. General characteristics of patients with NAFLD

	NAFLD group (n=250)	Control group (n=110)	P value
Male	186 (74.4%)	77 (70%)	
Female	64 (25.6%)	33(30%)	
Age	53.6±8.6	49.3±9.2	
Smoking	102 (40.8%)	48(43.6%)	0.18
Obesity	158 (63.2%)	21(19%)	<0.001
BMI	31.8±3.2	26.2±3.8	<0.001
Waist circumference	100.8±8.9	89.5±3.4	<0.001
Diabetes mellitus	118(47.2%)	21 (19.1%)	<0.001
Physical activity METs-hour/week	162.7±68.4	160.5±66.8	0.22
Dyslipidemia	132(52.8%)	36(32.7%)	<0.001
Metabolic syndrome	114 (45.6%)	19(17.3%)	<.0001
Chronic kidney disease	22(8.8%)	8(7.3%)	0.83
Arterial hypertension	67(26.8%)	11(10%)	<0.001

The results of the laboratory tests on the examined subjects are presented in Table 2.

TABLE 2. Laboratory test results

Characteristics	Number (n=250)	Control group (n=110)	P value
ALT, IU/L	44.6±27.8	25.6±11.7	<0.001
AST, IU/L	41.2±26.4	20.8±6.4	<0.001
GGT, IU/L	61.3±33.8	30.3±6.9	<0.001
Fasting glucose, mg/dL	98.7±24.2	78.6±18.4	<0.001
HbA_1C, %	6.1±2.1	5.36±1.8	0.014
Total Cholesterol, mg/dL	208.6±38.1	192.3±22,4	0.92
LDL Cholesterol, mg/dL	126.4±30.4	124.7±20.6	0.87
HDL Cholesterol, mg/dL	48.3±8.9	49.8±6.9	0.45
Triglycerides, mg/dL	152.2±57.7	118.4±15.7	<0.001
Albumin (g/dL)	3.4±0.22	4.1±0.42	<0.001
Platelet count ($x10^3$/mm)	346.4±68.5	190.3±52.7	<0.001

As laboratory studies have shown, NADL patients exhibited significantly higher levels of transaminases, triglycerides, glucose, and platelets, as well as significantly lower levels of albumin.

In 250 patients with NAFLD, we studied clinical indices and ultrasound grading of this disease.

Grade 1 had 88(35.2%) patients; grade 2 – 104(41.6%) and grade 3 – 58(23.2%) patients.

The results of the clinical indices are distributed according to grades of NAFLD, as shown in Table 3.

TABLE 3. Clinical indices according to NAFLD grading

	FLI	HSI	LAP	P value
Grade 1 (n=88)	39.6±4.38	32.1±2.6	30.7±2.2	<0.001
Grade 2 (n=104)	59.8±11.4	46.8±10.3	38.5±7.6	<0.001
Grade 3 (n=58)	82.8±5.6	52.7±3.9	50.1±3.8	<0.001

Abbreviations: FLI, fatty liver index; HSI, hepatic steatosis index; LAP, lipid accumulation products.

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease and affects approximately 1.7 billion people worldwide. NAFLD includes a broad spectrum of disease activity, from simple steatosis to non-alcoholic steatohepatitis (NASH).[22,23] Simple steatosis has a benign and potentially reversible course; however, NASH can progress to advanced

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fibrosis, cirrhosis, and hepatocellular carcinoma. The diagnosis of NAFLD remains underrecognized, as most patients are asymptomatic until late stages of the disease.[24] So, early diagnosis of NASH and accurate staging of fibrosis risk are critical for better stratification, monitoring, and targeted management of at-risk patients. Liver biopsy is the gold standard for diagnosing NAFLD and the most accurate tool for grading fibrosis; however, it is an invasive procedure that carries a risk of complications. Therefore, the development of non-invasive diagnostic tools is significant.

In this study, we analyzed general information, biochemical indices, and risk factors associated with NAFLD. We searched for significant and relevant diagnostic indices from physical examination data in the Georgian population.

We analyzed the health data of 360 subjects to investigate the risk factors associated with the development of NAFLD, comprising 250 patients with NAFLD and 110 subjects without NAFLD. We found that Obesity, BMI, waist circumference, diabetes mellitus, arterial hypertension, dyslipidemia, and metabolic syndrome were risk factors for the occurrence of NAFLD.

As for the results of laboratory research, AST, ALT, GGT, Glucose, Triglycerides, Albumin, and Platelet count were reliably associated with the development of NAFLD.

Obesity is a key factor in the development of NAFLD. In the present study, we found that obesity and BMI were the best predictors of NAFLD, a finding supported by several other studies.[25,26]

Dyslipidemia is also a risk factor for the development of NAFLD. In this study, Triglycerides were the most substantial risk factor for NAFLD.

We found that hypertension is one of the risk factors for NAFLD. In our study, about 27% of NAFLD patients had hypertension. NAFLD is associated with changes in arterial stiffness, myocardial remodeling, renal disease, and an increased risk of heart failure.[27,28]

Among the characteristics of Metabolic syndrome, hyperglycemia is most clearly biologically linked to the progression of NAFLD, with up to 75% of patients with type 2 diabetes suffering from NAFLD. In our study, the incidence of diabetes was 47.2%. Patients with NAFLD who have diabetes also have a higher prevalence of NASH and advanced fibrosis compared to non-diabetic patients with NAFLD.[29,30]

In patients with NAFLD, we found a significant decrease in albumin levels: 3.4±0.22 vs 4.1±0.42. The role of serum albumin in the development of NAFLD has not yet been precisely established. However, Ge, Liao, et al. studied 23 patients with NAFLD and found that the binding capacity of albumin, including for metals and fatty acids, decreases before the total plasma albumin or bilirubin concentration shows abnormalities. In addition, it was demonstrated that the albumin conformation in NAFLD patients does change.[31]

Interesting data was obtained from the CBC study, which revealed a significant increase in platelets compared to the control group: 346.4±68.5 vs 190.3±52.7. Platelets are involved in different models of liver damage. Scientific evidence supports the hypothesis that platelets are involved in the pathophysiology of NAFLD/NASH, primarily by exerting proinflammatory and profibrotic effects. NAFLD represents the result of chronic inflammation's impact on the liver. The inflammatory state is mainly due to metabolic imbalance, leading to metabolic syndrome, obesity, insulin resistance, and type 2 diabetes. Lipid species induce inflammation and activate both infiltrating and resident immune cells. Platelets are involved in pathological processes such as chronic inflammation, atherothrombosis, and possibly fibrogenesis.[32,33]

One of the aims of our study was to investigate the correlation between clinical indices of NAFLD and ultrasound findings.

Ultrasound, as a non-invasive tool, plays a crucial role in the diagnosis of NAFLD. In comparison to other non-invasive imaging techniques, ultrasound has comparable sensitivities, greater ease of use, availability, and lower cost in screening for moderate to severe degrees of NAFLD. Williams et al used ultrasound to screen asymptomatic individuals in the general population without known liver disease; they found non-alcoholic fatty liver disease to be more prevalent than previously reported. All individuals who had ultrasound findings suggestive of fatty liver had a liver biopsy to confirm their diagnosis. Using ultrasound as a screening tool, they found that NAFLD was present in 46% of the population.[34] However, ultrasound has some limitations. Multiple studies have shown that ultrasound underestimates the prevalence of hepatic steatosis when less than 20% of the liver is affected by steatosis. The sensitivity for detecting mild degrees of steatosis is low, ranging from 55% to 90%.[35,39]

Our study data showed that clinical indices, including FLI, HIS, and LAP, have high sensitivity and specificity in diagnosing NAFLD and are significantly correlated with ultrasound data. It was also revealed that clinical indices correlate with the severity of the NAFLD determined by ultrasound examination, i.e., the degree of fatty infiltration.

Georgian Biomedical News
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CONCLUSIONS

In conclusion, we analyzed the medical examination information of the Georgian population, and the results showed that the occurrence of NAFLD is closely related to METs. Individuals should manage their BMI, blood glucose levels, hypertension, blood lipids, and liver function tests to prevent the development of NAFLD. Our study verifies the accuracy of clinical indices, including FLI, HIS, and LAP, for diagnosing NAFLD as assessed using ultrasound examination. Therefore, clinical indices, combined with ultrasound, are a powerful and valuable diagnostic tool for detecting NAFLD, which can reduce the need for MRI and liver biopsy.

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