BIOCHEMICAL SCORING SYSTEM FOR DIAGNOSING NONALCOHOLIC STEATOHEPATITIS

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Abstract

Background: *Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of conditions ranging from simple steatosis to steatohepatitis, advanced fibrosis, and end stage liver disease. Despite the high prevalence and severity of hepatic illness, NAFLD remains underdiagnosed, because of few symptoms, lack of accurate laboratory markers.*

Objective: *To evaluate a biochemical score for diagnosing non-alcoholic steatohepatitis.*

Methods: *An observational, cross sectional study was carried out for a period of two years in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. 43 patients of Non-alcoholic fatty liver disease (NAFLD) attending at department of Hepatology were selected and underwent for biochemical investigations and liver biopsy with NAFLD Activity Score (NAS).*

Results: *A biochemical score (TAAG score) assigned 1 point for each parameter (fasting serum triglyceride >ULN, alanine aminotransferase >ULN, AST/ALT ratio (AAR) ≤1 and gamma-glutamyl transferase >ULN) was evaluated. TAAG score ≥3 was present in 32.5% of study population and 40% of NASH patients. It had a sensitivity of 40%, specificity 26% and AUROC 0.54.*

Conclusion: *Biochemical scoring system comprising traditional biomarkers did not significantly predict NASH. Biopsy is the only way to estimate steatohepatitis and/or fibrosis.*

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Introduction:

NAFLD encompasses a histological spectrum ranging from simple steatosis to steatohepatitis, advanced fibrosis and inflammatory changes.[1] NAFLD is an acquired metabolic stress-induced liver disease associated with insulin resistance (IR) and genetic susceptibility, sharing histological similarities with alcoholic liver disease (ALD) in the absence of substantial alcohol consumption or other causes of liver disease[2]. Two broad types are recognized-simple steatosis is typically stable while non-alcoholic steatohepatitis (NASH) is characterized by significant cell injury and the potential for progression to cirrhosis[3].

Fatty liver may be diagnosed if liver echogenicity exceeds that of renal cortex and spleen and there is attenuation of the ultrasound wave, loss of definition of the diaphragm, and poor delineation of the intrahepatic architecture. However this finding is not specific and cannot be used to diagnose NASH. Its sensitivity range from 60-100% and its specificity from 77-95% in detecting fatty infiltration of the liver[4]. A complete diagnosis of fatty liver disease ideally should define the histology, including the stage and grade of the disease as well as its etiology.

ALT is a marker of hepatic steatosis or hepatic[5] and NASH has been associated with slight elevation of liver enzymes mostly ALT and Gamma-glutamyl transferase (GGT)[6]. Patient typically present with asymptomatic serum aminotransferase elevations of 2-3 times the normal[7].

The AST/ALT ratio is approximately 0.8 in normal subjects. The AST is greater than the ALT in alcoholic hepatitis and a ratio greater than 2:1 is highly suggestive of this disorder. A ratio >1.0 may also suggest the presence of cirrhosis in patients with chronic viral hepatitis[8]. While the AST/ALT ratio lower

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than 1 is highly suggestive of nonalcoholic steatohepatitis[9].

Excess deposition of fat in the liver is associated with an elevated serum GGT and insulin resistance[10]. An increased GGT level is a risk factor for advanced fibrosis in NAFLD[11] and, with weight loss, a decrease in GGT activity is predictive of improved lobular inflammation and fibrosis of liver.

Hepatic steatosis is a manifestation of excessive triglyceride accumulation in the liver. The major sources of triglycerides are from fatty acids stored in adipose tissue and fatty acids newly made within the liver through de novo lipogenesis[12].

The 'gold standard' for the diagnosis of NASH is liver biopsy, which allows us to differentiate simple steatosis from NASH[13]. However, there are practical limitations, including costs and risks. Importantly longer cores are needed for accurate fibrosis staging[13].

The view of this study was to develop a biochemical score to differentiate NASH from steatosis in NAFLD and correlate with NAFLD activity score (NAS).

Methods:

It was an observational, cross sectional study. The study was carried out for a period of 2 years in Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Patients of NAFLD attending at Hepatology department were selected as study population. We took 43 NAFLD patients for biochemical parameters, liver biopsy and NAS score evaluation in considering the exclusion and inclusion criteria. NAS score was constructed according to Kleiner et al. (2005). In our study we evaluated a biochemical score (TAAG score) assigned 1 point for each parameter (fasting serum triglyceride >ULN, ALT>ULN, AST/ALT ratio (AAR) d"1 and GGT >ULN). With a total of 4 possible points of TAAG score, we assessed and correlate cut-off points > 3 with NAS (NAFLD activity score) obtained from liver biopsy.

Patient's inclusion criteria was ultrasonographic evidence of fatty liver and exclusion criteria were significant alcohol intake (more than 20gm/day),viral hepatitis (HBV, HCV), pregnancy, co-morbid conditions (COPD, CRF, cardiac failure), hypothyroidism, consumption of drugs causing fatty change in liver (steroid, oral contraceptive pill, tamoxifen, amiodarone, diltiagem, protease inhibitor).

All data were collected from structured questionnaire and analyzed by SPSS 16 software. Qualitative data was analyzed by Chi-square test and quantitative data by student's t-test. P values below 0.05 considered as statistically significant.

Ethical consideration:

Ethical clearance for the study was taken from the Institutional Review Board of BSMMU prior to the commencement of this study.

Results:

We evaluated 43 patients (26 female, male 17). Patient's age, body weight, height, BMI, waist, systolic and diastolic blood pressure, ALT, AST, AST to ALT ratio, GGT, fasting blood sugar, 2 hr after breakfast, fasting serum lipid profile were analyzed. BMI and waist circumference was calculated according to Western Pacific Region Office of WHO 2000 criteria and International Diabetes Federation criteria 2006 for the South Asians respectively. We grouped the study population (n=43) into non-NASH fatty liver (NNFL) and nonalcoholic steatohepatitis (NASH). NNFL was present in 23 patients and NASH was in 20 patients.

Table-I

Baseline characteristics of study populations (n = 43).

Variables	Mean ± SD
Age (years)	41.1 + 10.5
Weight (Kg)	67.8 ± 10.3
Height (cm)	157.5 ± 8.6
BMI Kg/m^2	27.3 ± 3.0
Waist circumference (cm)	93.0 ± 6.9
SBP (mm Hg)	120.0 ± 21.7
DBP (mm Hg)	80.7 ± 8.2
ALT (U/L)	48.6± 25.4
AST (U/L)	40.0 ± 25.0
AST/ALT (AAR)	0.8 ± 0.3
GGT	50.0±34.9
FBS (mmol/L)	5.5 ± 1.3
2hrABF (mmol/L)	7.9 ± 1.7
Total Cholesterol (mg/dl)	212.5 ± 43.6
TG (mg/dl)	253.1 ± 202.7
HDL (mg/dl)	37.1 ± 9.8
LDL (mg/dl)	132.4 ± 39.7

Data is expressed as Mean ±SD. SD, Standard deviation.

Table-II

Clinical and laboratory characteristics of study population (n= 43).

Variables	NNFL (NAS 0-4) (mean ± SD)	NASH (NAS >5-8) (mean ± SD)	P value by Independent samples T test
Age (years)	39.2±9.5	43.2 ± 11.3	0.22
Weight (Kg)	66.7±9.7	69.1±11.0	0.45
Height (cm)	156.3±8.6	159 ± 8.5	0.31
BMI Kg/m^2	27.4±3.3	27.2± 2.7	0.88
Waist circumference (cm)	92.3±5.6	93.8 ± 8.3	0.53
SBP (mm Hg)	117.1±9.8	128.2± 15.2	0.009
DBP (mm Hg)	79.3±8.56	82.2 ± 7.69	0.24
ALT (U/L)	46.4±23.7	51.1±27.7	0.55
AST (U/L)	43.3±32.1	36.1±12.7	0.32
AST/ALT	0.9±0.3	0.80±0.3	0.27
GGT	46.5±29.4	58.3±40.2	0.28
FBS (mmol/L)	5.2±1.3	5.9±1.1	0.74
2hrABF (mmol/L)	7.7±1.4	8.2±2.0	0.43
Total Cholesterol (mg/dl)	209.8±41.4	215.5±46.9	0.67
TG (mg/dl)	262.7±264.6	242.1±97.5	0.73
HDL (mg/dl)	38.0±8.6	36.1±11.2	0.54
LDL (mg/dl)	131.4±37.5	133.4±42.8	0.87

p value was determined by Independent-Samples T test.

In the study 46.5% patients were NASH (M 40%, F 60%). 32.5% NASH patients were in metabolic range. Mean fasting serum TG level was 253 mg/dl. 41.86% NASH had TG level in metabolic range (e"150mg/dl). Serum TG e"ULN had sensitivity of 90% and specificity of 73% to identify NASH. AUROC is 0.59.

Serum ALT level above the ULN (65 U/L) was present in 25.5% NAFLD patients. High ALT presents with NASH was 54.5%. Serum ALT level above ULN not significantly correlates (p=0.53, chi-square test) in between NNFL and NASH. Serum ALT >ULN had sensitivity 30% and specificity 21.7% to identify NASH. AUROC is 0.56.

AST/ALT (AAR) ratio was d"1 in 79.0% NAFLD patients. AAR d"1 was present in 75% of NASH individuals and 82.6% of NNFL. In this study AAR not significant correlates (p=0.54, chi-square test) to identify NASH from NNFL. AST/ALT ratio (AAR) d"1 had sensitivity 75% and specificity 82.6% to identify NASH. AUROC was 0.39.

Serum GGT level (male 15-85U/L, female 5-55 U/L) above the ULN was 25.5% in study population. Mean serum GGT was 52 (34.9) U/L. Only 30% of NASH population presented with serum GGT above the ULN. Serum GGT >ULN had sensitivity 30% and specificity 21.7% to identify NASH. AUROC is 0.56.

TAAG score e"3 was present in 32.5% patients and 40% of NASH patients. TAAG score e"3 had a sensitivity of 40% and specificity of 26.1% not significantly correlates (p=0.33, chi-square test) to NASH prediction. ROC curve showing TAAG scoring system e"3 had sensitivity 40% and specificity 26.1% to identify NASH. AUROC is 0.54.

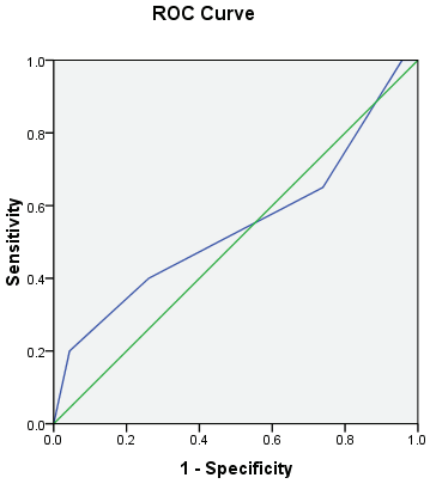

Fig.-1: *ROC curve showing TAAG scoring system e"3 had sensitivity 40% and specificity 26.1% to identify NASH. AUROC is 0.54.*

Discussion:

Our study aim was to create a biochemical scoring system to predict and identify NASH. Total 43 patients with ultrasonographic features of fatty liver were enrolled, who met the inclusion criteria.

Hypertriglyceridaemia was present in 58.1% and hypercholesterolaemia in 62.7% patients. Hypertriglyceridaemia has important role in the pathogenesis of NAFLD and previous studies from Asia also showed its significant contribution in NAFLD[14].

Serum liver enzyme abnormalities in NAFLD are primarily to elevations of ALT. The majority of elevations is mild (less than 5 times the upper limit normal) and exists in all degrees of NAFLD[15]. In our study, elevated ALT was present in 25.5% NAFLD, 30% of NASH population and raised ALT having no correlation (p=0.53, chi square test) in diagnosing NASH.

AST to ALT ratio (AAR) is usually less than 1 in NAFLD patients[2]. AAR > 1 can be an independent risk factor for advanced fibrosis in NASH according to some studies[4]. In our study, 79.0% patients presented with AAR d"1 having no correlation (p=0.54, chi-square test) in diagnosing NASH.

The role of GGT, as a marker for disease severity and diagnostics is still obscure in NAFLD. Serum GGT e"30 U/L is an adequate marker of NASH[15]. In our study, serum GGT >ULN did not have any correlation with (p=0.53, chi-square test) severity of liver disease. It is not an adequate marker for prediction of NASH.

We proposed a biochemical scoring system (TAAG score: triglyceride-alanine aminotransferase-aspartate aminotransferase to alanine aminotransferase ratio-gamma glutamyl transferase score) for diagnosing NASH, assigned 1 point for each of the following characteristics: TG >ULN (150 mg/dl), ALT >ULN (65 U/L), AST/ALT d"1 (AST ranges 15-37 U/L) and GGT >ULN (male 15-85 U/L, female 5-55 U/L). Biochemical investigations were done in the Department of Biochemistry, BSMMU. TAAG score e"3 out of 4 (sensitivity of 40%, specificity of 26%, p=0.25 by chi-square test, AUROC 0.54) was not a strong predictor of NASH.

Conclusion:

Biochemical scoring system comprising traditional biomarkers also did not significantly predict NASH. Biopsy is the only way to estimate steatohepatitis and/or fibrosis. We recommend liver biopsy in every patient of NAFLD with metabolic syndrome whenever possible.

References:

1. Adams L, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic fatty liver disease. Am J Gastroenterol. 2004; 99: 1316-1320. https://doi.org/10.1111/j.1572-0241.2004.30444.x PMid:15233671
2. Adams L, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic fatty liver disease. Am J Gastroenterol. 2004; 99: 1316-1320. https://doi.org/10.1111/j.1572-0241.2004.30444.x.PMid:15233671
3. Adams LA, Talwalkar JA. Diagnostic Evaluation of Nonalcoholic Fatty Liver Disease. J Clin Gastroenterol. 2006; 40: 34-38.
4. Andrea ER. Nonalcoholic Fatty Liver Disease. In: Mark F, Lawrence SF, Lawrence JB, editors. Sleisenger and Fordtran's gastrointestinal and liver disease: pathophysiology /diagnosis / management. 9th ed. Philadelphia: Elsevier; 2010. 1401-13. https://doi.org/10.1016/B978-1-4160-6189-2.00085-8
5. Caldwell SH, Al-Osmani AMS, Argo CK. Nonalcoholic fatty liver disease. In: Schiff ER, Maddrey WC, Sorrel MF, editors. Schiff's Disease of the liver. 10th ed. Philadelphia. Lippincott Williams & Wilkins; 2007. 1117-68
6. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology. 1999; 30: 1356-1362. https://doi.org/10.1002/hep.510300604. PMid:10573511
7. Angulo P, Hui JM, Marchesini G. The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007; 45: 846-854. https://doi.org/10.1002/hep.21496. PMid:17393509
8. Annurad E, Shiwaku K, Nogi A, Kitajima K, Enkhmaa B, Shimono K, Yamane Y. The New BMI criteria for Asians by the Regional Office for the Western Pacific Region of WHO are suitable for the screening overweight to prevent metabolic syndrome in Elder Japanese Workers. J occup Health. 2003; 45: 335-343.https://doi.org/10.1539/joh.45.335. PMid:14676412
9. Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH.Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. J. Hepatol. 2009; 51: 371-379. https://doi.org/10.1016/j.jhep.2009.03.019. PMid:19501928
10. Assy N, Kaita K, Mymin D.Fatty infiltration of liver in hyperlipidaemic patients. Dig. Dis. Sci. 2000; 45: 1929-1934.https://doi.org/10.1023/A:1005661516165. PMid:11117562
11. Bayard M, Holt J, Boroughs E.Nonalcoholic fatty liver disease. Am Fam Physician. 2006; 73: 1961-1968.
12. Bellentanis S, Scaglioni F, Marinom, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis. 2010; 28: 155-161.https://doi.org/10.1159/000282080.PMid:20460905

13. Bugianesi E, Manzini P, D'Antico S.Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology. 2004; 39: 179-187. https://doi.org/10.1002/hep.20023. PMid:14752836

14. Caldwell SH, Argo CK 2011, .Non-alcoholic Fatty Liver Disease and Nutrition. In: Dooley JS, Lok ASF, Burroughs AK, Heathcote EJ, editors. Sherlock's Diseases of the Liver and Biliary System. 12th ed. West Sussex: Wiley-Blackwell; 2011. 546-67.https://doi.org/10.1002/9781444341294.ch28

15. Fan JG, Zhu J, Li XJ, Chen L, Lu YS, Li L. Fatty liver and the metabolic syndrome among Shanghai adults. J Gastroenterol Hepatol. 2005; 20: 1825-1832. https://doi.org/10.1111/j.1440-1746.2005.04058.x. PMid:16336439

16. Pulzi FBU, Cisternas R, Melo MR, Ribeiro CMF, Malheiros CA, Salles JE. New clinical score to diagnose nonalcoholic steatohepatitis in obese patients. Diabetology & Metabolic Syndrome. 2011; 3: 3.https://doi.org/10.1186/1758-5996-3-3. PMid:21345221 PMCid:PMC3055806