

Original Article

Alanine Aminotransferase is a Predictor of NAFLD Activity Score for Diagnosing Non-alcoholic Steatohepatitis

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a metabolic disorder characterized by excessive triglyceride-accumulation in hepatocytes. NAFLD has a multifactorial etiology and a combination of environmental, genetic and metabolic factors play a role in the development of advanced disease. NAFLD consists of a wide spectrum of conditions, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) which can progress to cirrhosis and hepatocellular carcinoma (HCC). Despite the high prevalence and severity of hepatic illness, NAFLD remains under diagnosed, because of few symptoms, lack of accurate laboratory markers. The accurate diagnosis of NASH remains dependent on specific histological parameters in liver biopsy. Although liver biopsy remains the 'gold standard', there are practical limitations, including costs and risks. There is an increasing requirement for simple, less invasive, highly accurate and affordable screening tools. Alanine aminotransferase (ALT) has been proposed as a noninvasive and available marker for assessment of NASH. A hospital based observational study was carried out for a period of two years in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Data were analyzed by SPSS version 16. Statistical inference were done by estimating distribution, Chi-square test and student's

t-test respectively. Fifty (50) patients were analysed. Twenty five were NASH and twenty five were simple steatosis. ALT in NASH group were 97.0 ± 51.5 IU/L and in simple steatosis group were 55.5 ± 28.6 IU/L. In NASH group 64% of raised ALT had NASH. In Non-NASH group 16% of raised ALT had no NASH. There was significant difference in the NAFLD activity score for diagnosing NASH between elevated and normal ALT (*P* value 0.001).

Keywords: Nonalcoholic fatty liver disease, Alanine aminotransferase, NAFLD activity score (NAS), Non-alcoholic Steatohepatitis.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a metabolic disorder characterized by excessive triglyceride accumulation in hepatocytes.¹ NAFLD has a multifactorial etiology and a combination of environmental, genetic and metabolic factors play a role in the development of advanced disease. 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 NAFLD consists of a wide spectrum of conditions, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) which can progress to cirrhosis and hepatocellular carcinoma (HCC).⁴ 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.⁵

AST is a hepatic transaminase that plays a role in diagnosis of steatohepatitis. Up to 3.6% of people in the United

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States have asymptomatic increase in AST.⁶ In Asian studies, AST is considered as an independent marker for severity of hepatic fibrosis if it is at least twice as much as the maximum normal value.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

ALT is a marker of hepatic steatosis or hepatitis⁹ and NASH has been associated with slight elevation of liver enzymes¹⁰. Patients typically present with asymptomatic serum aminotransferase elevations of 2-3 times the normal¹¹. This was also explored by Pulzi et al 2011,¹² where majority had mild elevation but less than 5 times upper normal limit and exists in all degree of NAFLD. But Alam et al 2013 showed serum alanine aminotransferase levels were not able to predict NASH.¹³

NASH has been associated with slight elevation of liver enzymes mostly ALT and Gamma-glutamyl transferase (GGT)¹⁰. Excess deposition of fat in the liver is associated with an elevated serum GGT and insulin resistance.¹⁴ An increased GGT level is a risk factor for advanced fibrosis in NAFLD¹⁵ and with weight loss, a decrease in GGT activity is predictive of improved lobular inflammation and fibrosis of liver.

Liver biopsy remains the gold standard for the diagnosis of non-alcoholic steatohepatitis, which allows us to differentiate the simple steatosis from non-alcoholic steatohepatitis.¹⁶ There are practical limitations, including invasiveness, rare but potentially life-threatening complications like risk of bleeding, allergic reaction caused by local anesthetics, advanced age, poor acceptability, sampling variability and cost. Furthermore, due to the epidemic proportion of individuals with nonalcoholic fatty liver disease worldwide, liver biopsy evaluation is impractical, and non-invasive assessment for the diagnosis of non-alcoholic steatohepatitis and fibrosis is needed.

The alanine aminotransferase (ALT) is a useful tool for non-invasive and available marker for assessment of non-alcoholic steatohepatitis.

MATERIALS AND METHODS

It was a hospital based observational 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 conduct fifty 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) with steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2), and a separate fibrosis staging (0-4). The proposed NAS was the sum of steatosis, lobular inflammation, and hepatocellular ballooning. NAS is a strong scoring system. NAS of greater than or equal to 5 correlated with diagnosing of NASH and biopsy with scoring of 1 to 4 were diagnosed as simple steatosis. Patient's inclusion criteria were ultrasonographical evidence of fatty liver and patients from 18 to 60 years. Exclusion criteria were significant alcohol intake (AASLD Practice guideline 2018, significant alcohol consumption be defined as >21 standard drinks per week in men and >14 standard drinks per week in women over 2 years period), viral hepatitis (HBV, HCV), Wilson's disease, autoimmune liver diseases, hereditary haemochromatosis, primary biliary cirrhosis, cirrhosis of liver, pregnancy, co-morbid conditions (COPD, CRF, cardiac failure), hypothyroidism, consumption of drugs causing fatty change in liver (steroid, oral contraceptive pill, tamoxifen, amiodarone, diltiazem, protease inhibitor).

Liver biopsy was done in the department of Hepatology, BSMMU by Trucut liver biopsy needle 14 F 15cm. The tissue was processed at the Department of Pathology, by standard protocol in automatic tissue processor (BAVIMED 2050, BAVIMED Laborgeneratbau GmbH, Birkeau, Germany). The processed tissue was then properly embedded on the melted paraffin for making blocks and sections. The sections were stained with hematoxylin and eosin for microscopic examination. The ALT was measured by CI 4100 Architect plus autoanalyzer (Abbott, USA) by liquid reagent pyridoxal-5-phosphate. After receiving the liver biopsy report, they were grouped as non-alcoholic steatohepatitis and simple steatosis. Consecutive 25 non-alcoholic steatohepatitis patients and 25 simple steatosis patients confirmed by liver biopsy were included in this study.

STATISTICAL ANALYSIS

All data were presented as mean \pm SD and were analyzed by SPSS (version 16). The qualitative data were analyzed by Chi-squared test and the quantitative data were analyzed by student's t-test.

Performance of the test were assessed by sensitivity and specificity test. Statistically significant result were considered when p value <0.05.

ETHICAL CONSIDERATION

Ethical clearance for the study was taken from the Institutional Review Board of BSMMU prior to the commencement of this study. Approval paper was given by 75th IRB, BSMMU meeting held on 30th november 2014. (No. BSMMU/2014/13573).

RESULTS

Fifty (50) patients were analysed. Twenty five were NASH and twenty five were simple steatosis. ALT in NASH group were 97.0±51.5 IU/L and in simple steatosis group were 55.5±28.6 IU/L. Overall, twenty six (52%) had normal ALT.

Table-I: Distribution of the study patients by baseline characteristics (n=50)

Variables	Mean ±SD	Min-Max
Age (years)	40.8±9.2	25.0-60.0
Weight (kg)	64.5±9.2	45.0-90.0
Height (cm)	158.4±8.6	145.0-182.0
BMI (kg/m ²)	25.7±4.0	18.2-36.5
Waist circumference (cm)	95.9±9.5	76.0-122.0
Systolic blood pressure (mm of Hg)	129.2±14.6	100.0-160.0
Diastolic blood pressure (mm of Hg)	80.6±7.0	70.0-100.0
Platelet count (x10 ⁹ /L)	315.4±69.6	130.0-500.0
Fasting blood sugar (mmol/L)	6.2±2.6	3.7-15.3
2HABF (mmol/L)	9.5±4.4	5.1-24.7
Total cholesterol (mg/dl)	205.0±44.8	118.0-329.0
LDL (mg/dl)	122.8±39.2	42.0-212.0
HDL (mg/dl)	38.7±9.3	21.0-63.0
TG (mg/dl)	215.9±107.4	58.0-441.0
AST (U/L)	44.4±28.2	19.0-124.0
ALT (U/L)	76.2±47.4	19.0-259.0
AST/ALT	0.6±0.2	0.3-1.5
HOMA-IR	2.4±1.7	0.4-8.5
GGT (U/L)	61.7±41.4	12.0-209.0
Serum ferritin (µgm/L)	121.4±101.6	14.2-573.2

Table-II : Clinical and laboratory characteristics of study patients in two group (n=50)

Variables	NASH (n=25) Mean ±SD	Simple steatosis (n=25) Mean ±SD	P Value
Age (years)	41.8±10.7	39.7±7.5	0.425ns
Weight (kg)	65.6±8.6	63.3±9.7	0.444ns
Height (cm)	159.2±9.1	157.7±8.3	0.545ns
BMI (kg/m ²)	26.0±3.9	25.5±4.0	0.656ns
Waist circumference (cm)	97.9±9.0	93.9±9.8	0.139ns
Systolic blood pressure (mm of Hg)	129.8±16.9	128.6±12.2	0.774ns
Diastolic blood pressure (mm of Hg)	80.2±7.8	81.0±6.1	0.688ns
Platelet count (x10 ⁹ /L)	303.1±68.7	327.8±66.8	0.203ns
FBS (mmol/L)	6.6±2.8	5.9±2.2	0.330ns
2HABF (mmol/L)	10.0±4.2	9.1±4.7	0.478ns
Total cholesterol (mg/dl)	210.0±48.7	199.9±38.4	0.419ns
LDL (mg/dl)	126.0±40.5	119.6±36.7	0.561ns
HDL (mg/dl)	40.7±9.1	36.6±8.9	0.113ns
TG (mg/dl)	209.0±95.9	222.8±116.2	0.649ns
AST (U/L)	55.2±30.1	33.6±20.0	0.004s
ALT (U/L)	97.0±51.5	55.5±28.6	0.001s
AST/ALT	0.6±0.2	0.7±0.3	0.171ns
HOMA-IR	2.4±1.9	2.3±1.6	0.841ns
GGT (U/L)	73.6±48.6	49.9±25.4	0.035s
Serum ferritin (µgm/L)	139.4±124.5	103.5±69.9	0.214ns

In NASH group 64% of raised ALT had NASH . In Non-NASH group 16% of raised ALT had no NASH. There was significant difference in the NAFLD activity score for diagnosing NASH between elevated and normal ALT (P value 0.001).

ALT of the study patients

Mean ALT was found 97.0±51.5 U/L in NASH group and 55.5±28.6 U/L in simple steatosis group. The mean ALT was statistically significant (p value = 0.001) between two groups.

Table-III Distribution of the study patients according to ALT (n=50)

ALT (U/L)	NASH (n=25)		Simple steatosis (n=25)		P value
	N	%	n	%	
≤65	8	32.0	17	68.0	
66-100	6	24.0	6	24.0	0.001 ^s
>100	11	44.0	2	8.0	
Mean±SD	97.0	±51.5	55.5	±28.6	

s= significant

Table-IV : Distribution of NAFLD activity score or NAS score and ALT level (n=50)

		Liver biopsy	Simple steatosis	Non-alcoholic steatohepatitis	Total
ALT	Normal (<65)	n %	17 68.00%	8 32.00%	25 100.00%
	High (>65)	n %	8 32.00%	17 68.00%	25 100.00%
Total		n	25 50.00%	25 50.00%	50 100.00%

NAFLD activity score=NAS, Simple steatosis= NAFLD activity score 1-4, Non-alcoholic steatohepatitis (NASH)= NAFLD activity score 5 or more.

Table-V : Person correlation between NAFLD activity score and ALT level (n=50)

Statistics	Value	95% CI
Sensitivity	68.00%	46.50% to 85.05%
Specificity	68.00 %	46.50% to 85.05%
Positive Predictive Value	68.00%	53.05% to 79.98%
Negative Predictive Value	68.00 %	53.05% to 79.98%

Pearson correlation between NAFLD activity score (NAS) score and alanine aminotransferase (ALT) level is 0.321 which is statistically significant (p <0.05).

DISCUSSION

Non-alcoholic fatty liver disease has been shown to be independently associated with increased overall, liver-related and cardiovascular mortality.¹⁷ Although the liver-related but not cardiovascular, mortality is higher in patients with non-alcoholic steatohepatitis compared with simple steatosis.¹⁷ It is suggesting that progressive liver disease is mostly confined to non-alcoholic steatohepatitis.

Non-alcoholic fatty liver disease encompasses a spectrum of conditions ranging from simple steatosis to nonalcoholic

steatohepatitis (NASH), fibrosis and end stage liver disease by Ludwig et al 1980¹⁸. 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* lipogenesis¹⁹.

The progression of NAFLD to its advanced stages is associated with significant morbidity in approximately 20% of patients, including complications such as gastro-oesophageal varices, ascites, liver failure,

hepatopulmonary syndrome and encephalopathy.²⁰ Furthermore, greater than 20% of NAFLD patients may develop cirrhosis over their lifetime according to a study by Matteoni et al.²¹ Of the patients who develop cirrhosis, 30–40% may suffer liver-related mortality within a 10-year period.²² Therefore, recognizing patients with NASH and advanced fibrosis early in the disease spectrum is essential not only in managing but also in preventing further progression to cirrhosis and HCC, and its related complications.

In this study sixteen percent (16%) of patients with normal ALT levels had evidence of NASH. Contrarily, sixteen percent of the patients with elevated ALT did not have NASH. NASH was present in 59% of patients with normal ALT in a recent study by Fracanzani et al.²³, whereas the rate of NASH in patients with normal ALT was 2.9% by Lee et al.²⁴ This difference in rate of NASH in normal ALT patients may be multifactorial.

ALT level is often considered by many clinicians as an easily accessible surrogate marker for evaluating underlying liver disease activity and severity of liver injury.²⁵ Another study suggesting normal and elevated ALT levels do not correlate with the severity of NAFLD.²⁶

Serum ALT level above the 65 U/L was present in 48% of NAFLD patients. Mean ALT differed significantly in NASH patients (97.0 ± 51.5 U/L in NASH versus 55.5 ± 28.6 U/L in simple steatosis) (P value- 0.001). Alam et al 2013 showed serum alanine aminotransferase levels were not able to predict NASH¹³.

LIMITATION OF THE STUDY

The present study evaluated predictive values of serum ALT and NAFLD activity score (NAS) to distinguish between nonalcoholic steatohepatitis (NASH) and simple steatosis in patients with NAFLD. This study presents some limitations such as small number of patients (50 patient), they were not selected randomly and only selected those patients who attended OPD, so there may be selection bias. All patients were collected in this study from a single tertiary level hospital that may not represent general population of the country. So, current study suffered from lack of multi-centric different ethnic category of patients.

CONCLUSION

This study found a significant difference in the NAFLD activity score for diagnosing NASH between elevated and normal ALT (P-value 0.001).

RECOMMENDATION

Alanine aminotransferase (ALT) level can be used as a non-invasive marker for the diagnosis of nonalcoholic steatohepatitis in non-alcoholic fatty liver disease patients.

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