Transaminases and Gamma Glutamyl Transpeptidase for Detecting Nonalcoholic Steatohepatitis and Fibrosis in Nonalcoholic Fatty Liver Disease

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Abstract:

Background: Nonalcoholic steatohepatitis (NASH) and advanced fibrosis are the spectrum of nonalcoholic fatty liver disease (NAFLD) that may progress to cirrhosis. **Objective:** We aimed to determine the detecting capacity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGT) for NASH and significant fibrosis. **Methods:** Demographic and laboratory data of 502 sonologically diagnosed NAFLD patients were retrospectively analysed. Area under receiver operating characteristics curve (AUROC) was performed for NASH and fibrosis score ≥2 (significant fibrosis) with ALT, AST and GGT of 233 biopsied patients. **Results:** Of 502 patients ALT, AST and GGT was elevated in 252 (50.1%), 184 (36.7%) and 138 (27.4%) respectively. There was no difference in histological activity and fibrosis score between normal and elevated ALT and AST. Forty two (40.2%) NASH and 23(20.2%) significant fibrosis had normal ALT level. GGT was differed in NASH and Non NASH ($p < .005$) and between significant fibrosis ($p < .01$) and insignificant fibrosis. To detect NASH AUROC curve of GGT was 67.5%, whereas of ALT and AST was 55.2% and 55.7%. For significant fibrosis AUROC curve of ALT, AST and GGT was 44, 50 and 68.4 % respectively. GGT level of 39.5 U/L could detect NASH with a 63% sensitivity and 65% specificity irrespective of sex. GGT 40.5U/L had 60% sensitivity and 59 % specificity to detect significant fibrosis. For fibrosis ≥2 AUROC curve was 75.4% in male. **Conclusion**: No optimal ALT and AST level could detect NASH and fibrosis. GGT level of 40 U/L had a better detecting capacity for NASH and fibrosis especially in male.

Keywords: Fatty liver; NAFLD, NASH, Fibrosis, ALT, AST; GGT .

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Introduction:

Nonalcoholic fatty liver disease (NAFLD), which may present as simple fatty liver, steatohepatitis (NASH) or even cirrhosis [1, 2] represents the most common cause of chronic liver disease in industrialized countries [3, 4]. Prevalence of NAFLD in adult Asian both in developing and developed population has been increasing. The figures vary from 9 to 30% in Japan, 5-24% in China, 5 to 28% in India, 18% in Korea, 30% in Malaysia and 5% in Singapore[5]. Transaminases, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are indicators of hepatocellular injury. Several studies demonstrated that high levels of ALT are correlated with a higher risk of NASH[6, 7]. However, some studies have shown that patients with normal ALT levels may also have histological features of NASH and be at risk for disease progression [8, 9]. AST or ALT level greater than twice the upper limit of normal had a positive predictive value (PPV) for bridging fibrosis of 21% and a negative predictive value (NPV) of 93% [10]. An AST greater than twice the upper limit of normal was also independently predictive of portal or bridging fibrosis in an Asian study of 60 patients with NAFLD [11]. However, other studies have failed to confirm an association between simple aminotransferase levels and degree of fibrosis in patients who have NAFLD [12, 13, 14]. Furthermore, studies comparing NAFLD patients who had persistently raised ALT levels to those who had persistently normal ALT levels found no difference in the

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prevalence of advanced fibrosis and cirrhosis between the groups [15, 16]. The association between aminotransferase levels and histological changes are therefore inconsistent.

The hydrolysis of glutathione is mainly done by gamma glutamyl trans¬peptidase (GGT), which is a membrane bound glycoprotein and it also catalyses the transfer of the glutamyl groups from one peptide to another [17]. The GGT activity is considered as a sensitive index of the hepatobiliary dysfunction than alkaline phosphatase, due to its presence in the microsomes and the plasma membranes of hepatocytes [18]. The serum gamma glutamyl transpeptidase levels rise and return to normal levels later in the liver diseases than the transaminases levels. So, the estimation of GGT is of some value in chronic hepatitis, when the values persist in high levels. The gamma glutamyl transpeptidase level may rise more than any other enzymes and it persists for prolonged periods. The chronic hepatitis which is caused by the hepatitis B and C viruses is associated with high GGT levels, which can be used as a noninvasive diagnostic marker and as a predictor of fibrosis[19, 20]. We have designed this study to differentiate clinical,biochemical character of NAFLD patients in normal and elevated ALT level and histological changes in elevated and normal ALT, AST and GGT level. Detecting capacity of ALT, AST and GGT for NASH and significant fibrosis were also studied.

Methods:

Study population:

Records of a number of total 502 adult patients with NAFLD were available in the Department Hepatology of the Bangabandhu Sheikh Mujib Medical University between January 2009 and July 2013 retrospectively. Diagnosis of fatty liver by ultrasonography was defined by the presence of at least two or three abnormal findings including diffusely increased echogenicity ("bright") liver with liver echogenicity stronger than kidney or spleen and either deep attenuation of ultrasound signal or vascular blurring. In particular, all patients were negative for hepatitis B surface antigen and antibodies against hepatitis C virus (anti-HCV), they reported no alcohol use or a weekly alcohol use <210 g for male patients and <140 g for female patients. They were not on any potentially hepatotoxic drug or agent that could give rise to elevated enzymes or fatty change. Autoimmune hepatitis, Wilsons disease, Haemochromatosis and hypothyroidism were excluded by clinical evaluation and relevant laboratory investigation. Disease of bile duct, lungs, pancreas, brain, gall bladder, kidneys and the heart muscles were excluded by clinical evaluation and imaging because these may be reason of elevated GGT level 21.

Clinical and Laboratory Data:

Clinical and laboratory information were obtained from 502 eligible medical records. Demographic and baseline data ; age , sex and body mass index (BMI), laboratory information on levels of ALT, AST and GGT, total serum cholesterol and triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), fasting blood sugar (FBS) were recorded. ALT and AST level of 40 U/L and GGT level of 65U/L for male and 40U/L for female was considered as normal. Metabolic syndrome was defined according to Asian criteria 22, and three of the five listed criteria were considered: waist circumference ≥ 80 cm for women and ≥ 90 cm for men, serum triglyceride ≥ 150 mg/dL (1.7 mmol/L), serum high-density lipoproteins (HDL) cholesterol < 50 mg/dL (1.3 mmol/L) for women and < 40 mg/dL (1 mmol/L) for men, elevated blood pressure (systolic blood pressure ≥ 130 and or diastolic blood pressure ≥ 85 mmHg or drug treatment for hypertension) and fasting plasma glucose concentration ≥ 100 mg/dL (5.6 mmol/L) or drug treatment for diabetes. Obesity was defined as BMI ≥ 25 kg/m2 and < 25 kg/m^2 was defined as nonobese.

Histological assessment:

Liver histopathology reports of the 233 patients were available. The diagnosis of NASH was based on the criteria of Brunt et al [23], as modified by Kleiner et al [24]. In this scoring system, the degree of disease activity in NAFLD was evaluated using the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocyte ballooning (0-2); therefore, the score ranged from 0 to 8. A NAS of 5 or more was diagnosed as "definitive NASH", a NAS of <5 considered as non-NASH fatty liver (NNFL). The hepatic fibrosis

staging was as follows: 0 = no fibrosis; 1 = zone 3 fibrosis only; 2 = zone 3 and portal/ periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis.

Statistical analysis:

Statistical analyses were performed by SPSS® for Windows® ver 15 (SPSS Inc., Chicago, IL, USA). The results are presented as the mean ± SD for the quantitative data and as numbers or percentages for the categorical or qualitative data. The statistical differences in the quantitative data were assessed using a *t* test. Receiver operating characterstics (ROC) curve was built to explore sensitivity and specificity. The qualitative data were compared using the x^2 test. For all of the tests, significance was achieved at $P < 0.05$.

Results:

We found the documents of 502 patients who fulfilled the criteria of fatty liver. Females were dominating 297 (59.2%) and males were 205 (40.8%). Mean age of the study population was 40.7 ± 10.0 years, most of the populations 343 (68.4%) were of 31 to 50 years of age. According to Asian criteria 56 (11.2%) had normal BMI, 72 (14.3%) were overweight, 270 (53.8%) were obese I and 104 (20.7%) were obese II. ALT, AST and GGT level was 53.0 ± 51.2, 44.1 ± 47.8 and 47.1 ± 33.5 U/L respectively. Fasting blood sugar was 5.7 ± 1.8 mmol /Lt, insulin resistance index was 1.8 ± 1.3 U/L, HDL level was 37.1 ± 8.7 mg/dl and triglyceride was 227 ± 132.2 mg/dl. Diabetic and hypertensive were 125 (25.8%) and 123 (26.9%) respectively. Waist circumference was high in 302 (69.6%), HDL level was low in 252 (65.6%) and TG was high in 319 (72.8%). Over all metabolic syndrome was prevailing in 272 (59.9%). ALT was elevated in 252 (50.1%), AST was elevated in 184 (36.7%) and GGT was elevated in 138 (27.4%) of the study population. Elevated ALT was positively correlated with AST and GGT level. ALT was elevated in younger age, male sex and higher triglyceride level (Table I). BMI, fasting blood sugar, lower HDL, insulin resistance index, metabolic syndrome didn't differed significantly in patient with elevated and normal ALT.

Table -1

Base line characteristics and differences with ALT level

Variable	ALT Normal <_ 40U/L N = 250	ALT Elevated > 40 U/L N = 252
Age (years) (mean ±SD)	42.0 ± 10.2	39.7 ± 9.8
AST U/L	31.2 ± 14.4	56.5 ± 63.4
GGT U/L	39.1 ± 30.3	58.6 ± 34.9
BMI Kg/m2	27.5 ± 3.8	26.9 ± 3.9
Insulin resistance index	1.8 ± 1.2	1.9 ± 1.4
Fasting blood sugar mmol/l	5.7 ± 1.6	5.7 ± 1.8
HDL- Cholesterol mg/dl	37.1 ± 8.8	37.0 ± 8.5
Triglyceride mg/dl	209.2 ± 117.5	242.7 ± 144.2
Waist circu mference cm	93.3 ±9.7	92.8 ± 10.5
Sex		
Male n (%)	73(35.8)	132(64.2)
Female n (%)	177(59.6)	120(40.4)
Hypertension no(%)	81 (59.6)	54 (40.4)
Metabolic syndro me no (%)	147(48.8)	154 (51.2)

Valus are shown is mean ± SD, no(%) statistica analysis was done with t-tast, x2 test: GGT= Gamma Glutamyl Transpeptidase , BMI = body mass index , AST = aspartate aminotransferase, ALT = alanine aminotransferase.

Histology and enzyme levels:

Histological evaluation was available in 233 cases; Non NASH was 14 (6.1%), border line NASH was 116 (49.8%) and definite NASH was 103 (44.2%). Grade I, II and III steatosis was 92(39.5%), 106 (45.5%) and 35 (15.0%). No ballooning was seen in 9(3.9%), grade I and II ballooning was in 175 (75.4%) and 48 (20.7%). Lobular inflammation was absent in 13 (5.6%), grade I, II and III was seen in 120 (51.5%), 95 (40.8%) and 5 (2.1%) cases respectively. Fibrosis < 2 was 173 (74.1%) and ≥ 2 was 60 (25.9%). Steatosis was very strongly differed (p= .001) in elevated and normal GGT level. Steatosis was signifi-

Table-II
Histological Differences with Transaminases and GGT level

Variable	ALT Normal	ALT Elevated	AST Normal	AST Elevated	GGT Normal	GGT Elevated
Steatosis	1.6±0.7	1.8±0.7*	1.6±0.7	1.9±0.7*	1.6±0.8	2.1±0.7**
Lobular Inflammation	1.4±0.6	1.4±0.6	1.4±0.6	1.4±0.6	1.5±0.6	1.6±0.5
Ballooning	1.2±0.5	1.1±0.4	1.2±0.5	1.2±0.4	1.2±0.5	1.2±0.4
NAS	4.2±1.2	4.4±1.3	4.2±1.2	4.5±1.2	4.2±1.2	4.9±1.0**
NASH N (%)	42(40.2)	61 (46.9%)	57(41.9%)	40(45.5%)	61(41.7%)	42(75.0%)***
Fibrosis	1.2±0.8	1.2±0.8	1.2±0.8	1.3±0.8	1.1±0.7	1.4±0.8*
Fibrosis ≥ 2 N (%)	23 (20.2%)	37(30.3%)	39(26.4%)	21 (28.1%)	31(14.1%)	29 (32.4%)**

Valus are shown is mean ± SD, no(%) statistica analysis was done with unpaired t-tast, x^2 test:
GGT= Gamma Glutamyl Transpeptidase , BMI = body mass index , AST = alanine aminotransferase,
ALT = aspartate aminotransferase.

cantly higher with elevated ALT and AST level also. Lobular inflammation and ballooning was not differed significantly in elevated and normal ALT, AST and GGT level. NAS was similar in ALT and AST level but it was differed with elevated and normal GGT level (p= .002). Fibrosis was similar in ALT and AST of normal and elevated level. Fibrosis was higher (p= .01) with elevated GGT level. Here only GGT was elevated with increasing NAS and fibrosis score. GGT was positively correlated with NAS (p= .01) and fibrosis (p=.01) but ALT and AST did not correlated with NAS and fibrosis. Forty two (40.2%) NASH and 23(20.2%) significant fibrosis was prevailing with normal ALT level. In normal AST level NASH was 57(41.9%) and significant fibrosis was 39(26.4%). In current status of normal GGT level NASH was 61(41.7%) and significant fibrosis was 31(14.1%).(Table II)

A receiver operating characteristic (ROC) curve express that GGT level of 39.5 U/L could detect NASH with 63% sensitivity and 65% specificity. The area under the ROC (AUROC) curve was 67.5%(Figure I). There were no significant difference in the level of GGT in male and female to detect NASH. The AUROC curve for ALT and AST to detect NASH was only 55.2% and 55.7%.

For diagnosing fibrosis ≥ 2 AST/ALT was not very useful because AUROC curve was only 55%, for AST it was 50% and for ALT it was 44%. GGT was better to detect fibrosis ≥ 2 with AUROC was 68.3% (Figure II). A value of GGT 40.5U/L had 60% sensitivity and 59 % specificity. In male it had strongest detecting capacity with AUROC curve was 75.4 % whereas in female it was 65.2%. Sensitivity for NASH and significant fibrosis decreased with progressively higher GGT level, while specificity for NASH and advanced fibrosis increased with progressively higher GGT level.

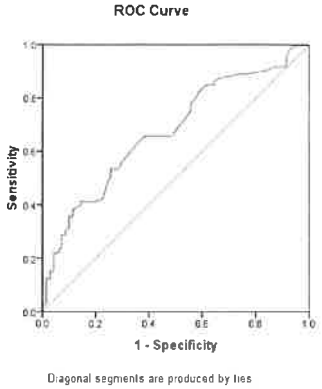

Fig-1: *Receiver operating characteristic curve to detect nonalcoholic steatohepatitis with GGT level*

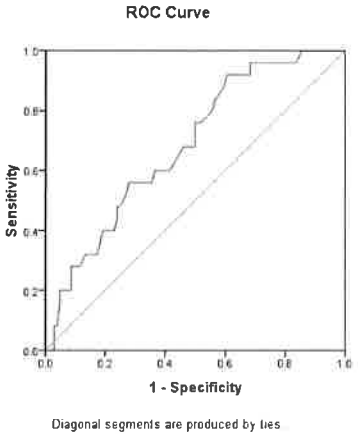

Fig-II: *Receiver operating characteristic curve to detect significant fibrosis with GGT level*

Discussion:

This retrospective series from Bangladesh is the largest study ever been published from Bangladesh where 502 NAFLD patients were included and 233 were biopsied. Female were 297 (59.2%) but sex did not influenced the development of NASH in this series. Our findings are in accordance with previous study from Bangladesh [25].Metabolic syndrome was present in 272(59.9%) but triglyceride (TG) was high in 319(72.8%).This could be justified as TG has long been considered as major factor in the development of NAFLD [6].

ALT was elevated in 252 (50.1%) and AST was elevated in 184 (36.7%) cases in our study. This number of patients with elevated ALT was lower than a recent study [26] because in that study ALT> 35 U/L was considered as elevated but we considered it >40 U/L, though the same kit was used for ALT and AST estimation. This is in accordance with recent reports from Malaysia where elevated ALT and AST was 47.8 % and 19.6% [27]. In another report from India observed that NAFLD with normal transaminases is common in Indian population also [5]. Elevated ALT was associated with elevated AST, GGT, younger age, male sex and higher triglyceride level. Study from USA with multi ethnic origin support these correlation of ALT with AST, age and sex as well as higher ALT is long been considered in male than female [28]. Progression of more than 20% of NAFLD to NASH and advanced fibrosis leads to significant morbidity and mortality with cirrhosis and hepatocellular carcinoma in the long run [29, 30]. For this reason traditionally elevated ALT and AST is considered for further clinical, radiological and histological evaluation. Our study shows dependence on elevated ALT level would lead to denial of detection 40.2% NASH and 20.2% significant fibrosis which is 41.9% and 26.4% for AST. It has been suggested by other reports that elevated liver enzymes does not strongly correlate with the level of liver injury, fibrosis or inflammation [12,31].Steatosis was differed with different values of ALT level in our study but not with ballooning, lobular inflammation and fibrosis level. Study from Iran 26 explored that ALT had correlation with steatosis but not with necroinflammation. Ballooning and fibrosis did not differ with ALT level in the study by Verma et al [28]. Wong et al. confirmed that ALT levels do not correlate well with metabolic and histological parameters in patients with NAFLD. Their results demonstrated that NASH and significant fibrosis can be found even among those with ALT below half of the upper normal limit[32].Another retrospective study on 233 obese women showed that patients with ALT < 19 U/L had less severe histopathologic findings. However, 23% and 5% of patients still had NASH and advanced fibrosis, respectively [33].

GGT was elevated in 138 (27.4%) of the study population. It could correlate with NAS and fibrosis, had better AUROC curve, sensitivity and specificity. The GGT activity is considered in previous study as a sensitive index of liver dysfunction due to its presence in the microsomes and the plasma membranes of hepatocytes[18]. GGT

levels rise and return to normal levels later in the liver diseases than the transaminases levels[34]. This could be the appropriate explanation for better AUROC curve of GGT for detecting NASH and advanced fibrosis. A lower level than presently accepted as normal that is of 40 U/L had better sensitivity and specificity for NASH and advanced fibrosis irrespective of sex.

Conclusion:

In conclusion, ALT and AST is not good detector for NASH in NAFLD. There are no ALT and AST cut-off value to predict disease severity, including NASH and significant fibrosis. GGT cut off value of 40 U/L is a better detector than ALT and AST for NASH and significant fibrosis. Fibrosis was better detected in male. NAFLD patients with high suspicion of NASH and significant fibrosis and GGT of > 40 U/L especially in male are the candidates for liver biopsy for further workup in NAFLD. We have got the limitations of single time estimation of liver enzymes and histology which may miss the dynamic status of histological activities and transaminases and summative effect fibrosis for long duration. All the patients were from single center and of same ethnicity and inter observer variability for histopathology could not elucidated.

References:

01. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346:1221–1231.

02. Rector RS, Thyfault JP, Wei Y, Ibdah JA.Non-alcoholic fatty liver disease and the metabolic syndrome: an update. World J Gastroenterol 2008; 14:185–192.

03. Argo CK, Caldwell SH. Epidemiology and natural history of non-alcoholic steatohepatitis. Clin Liver Dis. 2009; 13:511–531.

04. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004; 40:1387–1395.

05. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? J Gastroenterol Hepatol 2007; 22:788–93.

06. Amarapurkar DN, Amarapurkar AD, Patel ND, Agal S, Baigal R, Gupte P, et al. Nonalcoholic steatohepatitis (NASH) with diabetes: predictors of liver fibrosis. Ann Hepatol 2006; 5(1):30-3.

07. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007; 45(4):846-54.

08. Lee JY, Kim KM, Lee SG, Yu E, Lim YS, Lee HC, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in potential living liver donors in Korea: a review of 589 consecutive liver biopsies in a single center. J Hepatol 2007; 47(2):239-44.

09. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003; 37(6):1286-92.

10. Boza C, Riquelme A, Ibanez L, Duarte I, Norero E, Viviani P, et al. Predictors of nonalcoholic steatohepatitis (NASH) in obese patients undergoing gastric bypass. Obes Surg 2005;15(8):1148-53.

11. Tsang SW, Ng WF, Wu BP, Chow DA, Li ET, Wong TC. Predictors of fibrosis in Asian patients with non-alcoholic steatohepatitis. J. Gastroenterol. Hepatol. 2006; 21: 116-21

12. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999; 30:1356–1362.

13. Brunt EM; Neuschwander-Tetri BA; Oliver D; Wehmeier KR; Bacon BR. Nonalcoholic steatohepatitis: histologic features and clinical correlations with 30 blinded biopsy specimens. Hum Pathol 2004; 35(9):1070–82.

14. Chitturi S, Weltman M, Farrell GC, Donald Mc D, Liddle C, Samarasinghe D,et al. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASHwith C282Y but not with fibrotic severity. Hepatology 2002; 36(1):142–9.

15. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003; 37(6):1286–92.

16. Sorrentino P, Tarantino G, Conca P, Perrella A,Terracciano ML, Vecchione R et al. Silent non-alcoholic fatty liver disease-a clinical-histological study. J Hepatol 2004; 41(5):751–7.

17. Burt Ad, Day CP Pathophysiology of the Liver. In: Nacsween RNM, Burt AD, Portmann BC, Ishak KG, Schever PJ, Anthony PP eds. Pa¬thology of the liver. 4th edition. Edinburg: Churchill Livingstone. 2002; 67-105.

18. Penn R, Worthington DJ Is serum gumma-glutamyl transferase a misleading test. Br Med J 1983; 286: 531-35.

19. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poy¬nard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001; 357: 1069–75.

20. Hui AY, Chan HLY, Wong VWS, Liew CT, Chim AML, Chan FKL, et al. Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. Am J Gastroenterol 2005; 100: 616–23.

21. Goldberg DM. Structural, functional, and clinical aspects of gamma-glutamyltransferase. Crit Rev Clin Lab Sci 1980; 12 (1): 1–58.

22. Chitturi S, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. J Gastroenterol Hepatol 2007; 22: 778-787.

23. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander- Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposalfor grading and staging the histological lesions. Am J Gastroenterol

1999; 94: 2467-2474.

24. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp- Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41: 1313-1321.

25. Alam S, Alam SMNE, Chowdhury ZR, Alam M, Kabir J. Nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients of Bangladesh. World Journal of Hepatology 2013; 5: 281-88.

26. Khosravi S, Alavian SM, Zare A, Daryani NE, Fereshtehnejad SM, Daryani NE, et al. Non-alcoholic fatty liver disease and correlation of serum alanin aminotransferase level with histopathologic findings. Hepat Mon 2011; 11(6):452-8.

27. Goh SC, Ho ELM, Goh KL. Prevalence and risk factors of non-alcoholic fatty liver disease in a multiracial suburban Asian population in Malaysia. Hepatol Int 2013; 7: 548–554.

28. Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). Liver International 2013. doi: 10.1111/liv.12226

29. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999; 116: 1413–9.

30. Wong VW, Wong GL, Tsang SW, Hui AY, Chan AW, Choi PC et al. Metabolic and histological features of non-alcoholic fatty liver disease patients with different serum alanine aminotransferase levels. Aliment Pharmacol Ther 2009; 29: 387–96.

31. Alam S, Ahmad N, Mustafa G, Shrestha A, Alam AKMK, Khan M. Evaluation of normal or minimally elevated alanine transaminase, age and DNA level in predicting liver histological changes in chronic hepatitis B. Liver Int 2011; 31: 824–30.

32. Wong VW, Chan HL, Hui AY, Chan KF, Liew CT, Chan FK, et al. Clinical and histological features of non-alcoholic fatty liver disease in Hong Kong Chinese. Aliment Pharmacol Ther. 2004;20(1):45-9.

33. Kunde SS, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. Hepatology 2005;42(3):650-6.

34. Percy-Robb IW, Finlayson NDC.Clinical chemistry of liver disease. In: Shearman JC. Finlayson NDC Camilleri M, eds. Diseases of the gas¬trointestinal tract and liver. 3rd edition, London: Churchill Livingstone. 1977;735-61.