Original Article

Study of Serum Lipid Profile and Aminotransferases (ALT and AST) in Non-obese, Non-diabetic Nonalcoholic Fatty Liver Disease

ASM Mazedul Islam[1], MA Muttalib[2], Md. Nazrul Islam[3], Md. Rashedul Haque[4], Mohammad Rafiqul Hoque[5], Maknunnahar[6]

1. Associate Professor
   Department of Biochemistry
   Rangpur Medical College
2. Professor and Ex Head
   Department of Biochemistry,
   Mymensingh Medical College
3. Professor
   Department of Biochemistry
   Rangpur Medical College
4. Professor and Head
   Department of Biochemistry
   Rangpur Medical College
5. Associate Professor
   Department of Biochemistry
   Shaheed Syed Nazrul Islam Medical College
   Kishoreganj
6. Individual Researcher & MPH Course Student
   Rangpur Community Medical College

Correspondence to:
ASM Mazedul Islam
Associate Professor
Department of Biochemistry
Rangpur Medical College,Rangpur
Mobile: 01812404440
Email: dr.mazedul.islam@gmail.com

Abstract:

Background:
Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of liver diseases characterized by simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. It is the most common cause of cryptogenic cirrhosis. NAFLD is associated with obesity, insulin resistance, hypertension, type 2 diabetes mellitus, and dyslipidemia.

Objective:
The study aimed to assess the changes in lipid profile and aminotransferases (ALT and AST) in Non-alcoholic fatty liver disease (NAFLD).

Methods:
This observational cross-sectional study was carried out in the department of Biochemistry, Mymensingh Medical College in cooperation with the Centre for Nuclear Medicine and Ultrasound, Mymensingh from January 2012 to June 2013 after the approval of the protocol. A total of 120 subjects were included through purposive sampling, among them, 60 subjects were with fatty liver disease (group-I) and another 60 were without fatty liver (group-II) with ultrasonography. The sampling method was purposive (non-random). Relevant laboratory investigations were performed by using established methods to achieve the objectives.

Results:
The majority of the subjects with NAFLD (33.33%) were in 51-60 years (in Group-I). Mean serum total cholesterol, triacylglycerol, LDL-cholesterol, and HDL-cholesterol were 203.33±21.83, 184.41±33.31, 126.28±18.75, and 40.17±6.32 mg/dl in group-I and 167.65±9.83, 128.77±11.65, 101.74±9.29 and 40.16±3.55 mg/dl in group-II respectively. Significant differences were found in the case of total cholesterol ($p<0.001$), triacylglycerol ($p<0.001$), and LDL-cholesterol (<0.001), but no significant difference was found in the case of HDL-cholesterol ($p>0.05$). Serum ALT and AST were 49.43±16.98, 45.20±13.56 U/L in group-I and 32.83±4.54, 28.68±4.31 U/L in the group- II respectively. Significant differences were found in case of ALT ($p <0.001$) and AST ($p <0.001$).

Conclusion:
Nonalcoholic fatty liver disease is associated with dyslipidemia and with the elevation of serum ALT and AST.

Keywords: Non-alcoholic fatty liver, Dyslipidemia, Ultrasonography.

Introduction:

Non-alcoholic fatty liver disease is a clinicopathological condition characterized by fatty infiltration in the liver mostly in the form of triacylglycerols which exceeds 5% of liver weight. Histologically, it is similar to alcoholic fatty liver disease, but it occurs in the absence of excessive alcohol consumption (<30 g/day in men and <20 g/day in women). NAFLD represents a spectrum of liver damage ranging from simple steatosis to steatohepatitis, fibrosis, and cirrhosis.[1,2] It is now considered as the most common chronic liver disease in developed as well as developing countries. NAFLD is becoming a major public health problem due to the rising prevalence of obesity and type 2 diabetes mellitus.[3] Histologically, NAFLD is categorized into simple steatosis and nonalcoholic steatohepatitis (NASH). Simple steatosis has a benign prognosis but NASH may progress to fibrosis, cirrhosis, hepatocellular carcinoma, and even hepatic failure.[4] NAFLD is now considered as the most common cause of cryptogenic cirrhosis. NASH most likely causes 80% of cases of cryptogenic cirrhosis, which accounts for 10% to 20% of all cirrhosis.[5] NAFLD is associated with obesity, insulin resistance, hypertension, type 2 diabetes mellitus, and dyslipidemia. It is now regarded as the hepatic manifestation of metabolic syndrome.[6] The prevalence of dyslipidemia in NAFLD is highly variable, ranging between 4% and 90% in different studies.[7,8] Dyslipidemia has been reported in 20%-90% of patients with NAFLD.[9] Hypertria-

cylglycerolemia is strongly correlated with NAFLD. Raised triacylglycerol was found in 92.15% and raised cholesterol was observed in 47.05% of patients in a study by Taseer et al.[10] NAFLD is associated with insulin resistance and hyperinsulinemia even in non-obese subjects with normal glucose tolerance.[11] NAFLD is recognized as the hepatic component of metabolic syndrome which includes glucose intolerance, obesity, hyperlipidemia, and hypertension. The risk and severity of NAFLD increase with the number of components of metabolic syndrome that are present.[12] Insulin resistance is responsible for causing abnormalities in lipid storage and lipolysis which may induce increased flux of free fatty acids to the liver and cause fatty liver disease.[13] Many studies showed that insulin resistance plays an important role in the pathogenesis of NAFLD. Due to insulin resistance, there is increased fatty acid flux and de novo lipogenesis in the liver is increased resulting in increased TAG synthesis and production of TAG-rich VLDL.[14] Deregulation of lipid metabolism in fatty liver is accompanied by overproduction of very low-density lipoprotein (VLDL).[15] Most of the patients with NAFLD who come to medical attention are identified as a result of incidentally discovered elevated hepatic enzymes. The aminotransferases (ALT and AST) are sensitive indicators of hepatocyte injury. Mild to moderate elevation of ALT and AST are the most common and often the only laboratory abnormality found in NAFLD.[9] ALT is most closely associated with hepatic steatosis.[16] NAFLD patients usually have a ratio of AST to ALT of <1. This ratio can reverse if fibrosis develops.[1] However, many patients with NAFLD do not have elevated hepatic enzyme levels. In the Dallas Heart study, 79% of patients with hepatic steatosis had normal ALT levels.[17]

In Bangladesh, NAFLD has not been addressed sufficiently by the medical community. The prevalence of NAFLD and its potential risk factors have not been previously explored. A study at Bangabandhu Sheikh Mujib Medical University showed the prevalence of NASH is 42.4% among NAFLD, which is high and alarming for Bangladesh.[18] Dyslipidemia is a known risk factor for cardiovascular diseases. Elevated levels of serum ALT and AST indicate hepatocellular injury. So, we conducted this study to see the changes in lipid profile and aminotransferases (ALT and AST) in non-alcoholic fatty liver disease (NAFLD).

Methods:

This was an observational cross-sectional study carried out in the department of Biochemistry, Mymensingh Medical College in cooperation with the Centre for Nuclear Medicine and Ultrasound, Mymensingh. The study was conducted from January 2012 to June 2013 after the approval of the protocol by the ethical committee. During the study period, a total of 120 subjects were included through purposive sampling. Among them, 60 subjects between 20-60 years of age with fatty liver disease in ultrasonography were included as cases and 60 subjects of a similar age group with normal ultrasonography were included as controls. The sampling method was purposive (non-random). The subjects have a history of alcohol intake of more than 20 grams/day, history of diabetes mellitus or fasting hyperglycemia (FBG >126 mg/dl), obesity (BMI>30 kg/m^2), hypertension, positive serological finding for hepatitis B or C virus, pregnancy, ingestion of drugs known to produce hepatic steatosis including corticosteroids, oral contraceptive pill (OCP), methotrexate, etc, known case of hypothyroidism, nephrotic syndrome, chronic renal failure, Cushing's syndrome were excluded from the study. Subjects were clinically examined and questionnaires were filled up after the interview. With all aseptic precautions, 5ml of venous blood was drawn. Following centrifugation, serum was picked up for biochemical investigations. Laboratory investigations were carried out by using established methods.The data were processed and analyzed by computer software SPSS (Statistical Package for Social Science) version 11.5. The mean value of the findings was compared between the two groups. Categorical variables were analyzed by using students' unpaired 't-tests, p-value <0.05 was taken as the level of significance.

Results:

The majority of the subjects with NAFLD (33.33%) were in the age group of 51-60 years (Table-I).

Table-I: Age distribution of study subjects

Age in years	Group -I	Group –II
20-30	12(20%)	10(16.67%)
31-40	10(16.67%)	13(21.67%)
41-50	18(30%)	20(33.33%)
51-60	20(33.33%)	17(28.33%)

In group-I, mean serum total cholesterol, triacylglycerol, LDL-cholesterol and HDL-cholesterol were 203.33±21.83, 184.41±33.31, 126.28±18.75, and 40.17±6.32 mg/dl respectively and in group-II, 167.65±9.83, 128.77±11.65, 101.74±9.29 and 40.16±3.35 mg/dl respectively. By comparing group-I with group II, highly significant differences were found in the case of total cholesterol (p<0.001), triacylglycerol (p<0.001), and LDL-cholesterol (<0.001), but no significant difference was found in the case of HDL-cholesterol (p>0.05). (Table-II).

Table-II: Mean±SD of the lipid profile of the study subjects

Biochemical variables (mg/dl)	Group–I (n=60) Mean±SD	Group–II (n=60) Mean±SD	p-value
Total cholesterol	203.33±21.83	167.65±9.83	<0.001
Triacylglycerol	184.41±33.31	128.77±11.65	<0.001
LDL – C	126.28±18.75	101.74±9.29	<0.001
HDL – C	40.17±6.32	40.16±3.55	> 0.05

p-value < 0.05 = significant

Serum ALT and AST were 49.43±16.98, 45.20±13.56 U/L in group-I and 32.83±4.54, 28.68±4.31 U/L in group II respectively. By comparing between two groups, significant differences were found in the case of ALT (p <0.001) and AST (p <0.001) (Table-III).

Table-III: Mean±SD of aminotransferases (ALT and AST) of the study Subjects

Biochemical variables (U/L)	Group–I (n=60) Mean ± SD	Group–II (n=60) Mean ± SD	p-value
ALT	49.43±16.98	32.83±4.54	<0.001
AST	45.20±13.56	28.68±4.31	<0.001

p-value < 0.05 = significant

Discussion:

Nowadays, nonalcoholic fatty liver disease is a common hepatic disorder and may contribute to the burden of chronic liver disease. A considerable part of NAFLD patients may develop nonalcoholic steatohepatitis which can develop fibrosis and cirrhosis. A liver biopsy followed by histological examination was a very important part of diagnosis in many studies. However, being an invasive procedure, liver biopsy cannot be performed at large in patients with no significant liver disease. In our study, ultrasound proved NAFLD patients who were non-obese and non-diabetic, were considered as cases. Ultrasound has 80% sensitivity and 99% specificity.[19] There are only a few studies to show abnormalities of blood lipids and liver enzymes (ALT and AST) in apparently healthy subjects with NAFLD. Obesity and diabetes mellitus are well-known risk factors for NAFLD and obesity, diabetes, as well as hypertension, are associated with abnormalities of blood lipids and elevated ALT and AST. They may play an important role as confounding factors. Therefore, we studied lipid profile, ALT, and AST in non-obese and non-diabetic subjects who are incidentally diagnosed as NAFLD on ultrasonography. In our study, we measured serum lipid status by estimating TAG, total cholesterol, HDL cholesterol, and LDL cholesterol. We found a significant increase (p<0.001) in serum TAG, total cholesterol, and LDL-cholesterol but there was no significant difference in HDL-cholesterol (p>0.05) when compared to controls. In our study, significantly higher serum total cholesterol level was observed in subjects with NAFLD in comparison to controls. This finding was in agreement with the result of Gupte et al,[20] Taseer et al[10], Kim et al[21] However, the observation by Jin et al,[22] Luxmi et al,[23] and Wasfy et al[24] showed no significant difference in total cholesterol level between NAFLD subjects and controls. In our study, significantly higher serum triacylglycerol levels were observed in subjects with NAFLD in comparison to controls. Similar findings were observed by Taseer et al,[10] Luxmi et al,[23] Kim et al,[21] and Krishnan and Venkatraman.[25] Jin et al,[22] in a study conducted in China also observed that fatty liver positively correlated with increased plasma triacylglycerol levels. In our study, there was a significantly increased level of LDL-cholesterol in NAFLD subjects compared to controls. Similar findings were observed by Chawla et al,[26] Kim et al,[21] Krishnan and Venkatraman[25] and Luxmi et al.[23] The observation of our study was in disagreement with the study by Uchil et al[27] and Lee et al[28] who found no significant difference of LDL-cholesterol between NAFLD subjects and controls. Increased TAG, total cholesterol, and LDL-cholesterol might be due to the alteration of lipid metabolism of NAFLD subjects.

No significant differences in total cholesterol and LDL-cholesterol level were observed by some studies. These might be due to decreased synthesis of VLDL or blockage of VLDL secretion from the liver as a result of microsomal triacylglycerol transfer protein (MTTP) polymorphism.[29] There was no significant difference in HDL-cholesterol in NAFLD subjects as compared to controls in our study. This result was supported by the observation by Wasfy et al[24] This might be a result of the exclusion of obese and diabetic subjects from our study. However, significantly low HDL-cholesterol levels were observed in some studies.[21-22] The results of our study showed significantly high serum ALT and AST levels in NAFLD subjects in comparison to the controls. This finding resembled the observation by Krishnan and Venkatraman,[25] Kotronen et al[16] and, Angulo[1]. The aminotransferases (ALT and AST) are sensitive indicators of hepatocyte injury. In fatty liver, hepatocytes are loaded with TAG. Insulin increases intracellular fatty acids, lipid peroxidation, mitochondrial swelling, dysfunction increased lysosomal fragility, and impaired membrane integrity contributing to hepatocyte injury and causing the release of these enzymes from the damaged hepatocytes.[4] Our study was not in agreement with the study by Browning et al,[17] Taseer et al,[10] and Mofrad et al,[30] who found no significant difference in aminotransferases between NAFLD subjects and controls. These subjects had mild steatosis on liver biopsy without hepatocellular inflammation.

Conclusions:

Nonalcoholic fatty liver disease is a part of metabolic syndrome which is linked with significant cardiovascular morbidity and mortality. Nonalcoholic fatty liver disease is associated with dyslipidemia and elevated of serum ALT and AST. So, it is recommended to estimate serum lipid profile and ALT and AST for better management of patients with nonalcoholic fatty liver disease.

Limitations of the study:

The sample size was small, the sampling technique was purposive (non-random) and LDL cholesterol was calculated by Friedewald's equation in our study. Further studies with a large number of subjects, random sampling techniques, and direct estimation of LDL cholesterol by the colorimetric method may be planned to give a conclusive decision.

References:

1. Angulo P. Obesity and nonalcoholic fatty liver disease. Nutr Rev. 2007 Jun;65(6 Pt 2):S57-63. doi: 10.1111/j.1753-4887.2007.tb00329.x.
2. Fassio E, Alvarez E, Domнnguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. Hepatology. 2004 Oct;40(4):820-6. doi: 10.1002/hep.20410.
3. Ratziu V, Poynard T. Assessing the outcome of nonalcoholic steatohepatitis? It's time to get serious. Hepatology. 2006 Oct;44(4):802-5. doi: 10.1002/hep.21391.
4. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology. 2003 May;37(5): 1202-19. doi: 10.1053/jhep.2003.50193. Erratum in: Hepatology. 2003 Aug;38(2):536.
5. Grattagliano I, Portincasa P, Palmieri VO, Palasciano G. Managing nonalcoholic fatty liver disease: recommendations for family physicians. Can Fam Physician. 2007 May;53(5):857-63.
6. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes. 2001 Aug;50(8):1844-50. doi: 10.2337/-diabetes. 50.8.1844.
7. Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology. 1994 Oct;107(4):1103-9. doi: 10.1016/0016-5085(94) 90235-6.
8. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C,et al. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology. 2002 Feb;35(2):373-9. doi: 10.1053/ jhep.2002.30692.
9. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002 Apr 18;346(16):1221-31. doi: 10.1056/ NEJMra011775.
10. Taseer IH, Hussain L, Safdar S, Mirbahar AM, Ahmed I. Frequency of nonalcoholic fatty liver disease(NAFLD) and its biochemical derangements in type 2 diabetes patients. Pak J Med Sci. 2009; 25: 817-820. https://www.pjms.com.pk/ issues/oct-dec109/article/article22.html [Accessed 22nd June 2022]
11. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome.

Hepatology. 2003 Apr;37(4):917-23. doi: 10.1053/jhep.2003.50161.
12. Chung SA, Greenberg GR, Diamant NE. Relationship of postprandial motilin, gastrin, and pancreatic polypeptide release to intestinal motility during vagal interruption. Can J Physiol Pharmacol. 1992 Aug;70(8):1148-53. doi: 10.1139/y92-159.
13. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology. 1998 Apr;114(4):842-5. doi: 10.1016/s0016-5085(98)70599-2.
14. Harrison SA, Day CP. Benefits of lifestyle modification in NAFLD. Gut. 2007 Dec;56(12):1760-9. doi: 10.1136/gut.2006.112094.
15. Cali AM, Zern TL, Taksali SE, de Oliveira AM, Dufour S, Otvos JD, et al. Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. Diabetes Care. 2007 Dec;30(12):3093-8. doi: 10.2337/dc07-1088.
16. Kotronen A, Westerbacka J, Bergholm R, Pietilδinen KH, Yki-Jδrvinen H. Liver fat in the metabolic syndrome. J Clin Endocrinol Metab. 2007 Sep;92(9):3490-7. doi: 10.1210/jc.2007-0482.
17. 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 Dec;40(6):1387-95. doi: 10.1002/hep.20466.
18. Alam S, Noor-E-Alam SM, Chowdhury ZR, Alam M, Kabir J. Nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients of Bangladesh. World J Hepatol. 2013 May 27;5(5):281-7. doi: 10.4254/wjh.v5.i5.281.
19. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002 Sep;123(3):745-50. doi: 10.1053/ gast. 2002. 35354.
20. Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S,et al.. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. J Gastroenterol Hepatol. 2004 Aug;19(8):854-8. doi: 10.1111/j. 1440-1746.2004.03312.x.
21. Kim HJ, Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW,et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. Arch Intern Med. 2004 Oct 25;164(19):2169-75. doi: 10.1001/ archinte.164.19.2169.
22. Jin HB, Gu ZY, Yu CH, Li YM. Association of nonalcoholic fatty liver disease with type 2 diabetes: clinical features and independent risk factors in diabetic fatty liver patients. Hepatobiliary Pancreat Dis Int. 2005 Aug;4(3):389-92.
23. Luxmi S, Sattar RA, Ara J . Association of nonalcoholic fatty liver with type 2 diabetes mellitus. JLUMHS. 2008; 7 (3): 188-193. https://pesquisa.bvsalud. org/portal/resource/pt/emr-197938. [Accessed 22nd June 2022]
24. Alam S, Fahim SM, Chowdhury MAB, Hassan MZ, Azam G, Mustafa G, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in Bangladesh. JGH Open. 2018 Mar 30;2(2):39-46. doi: 10.1002/jgh3.12044.
25. Krishnan A, Venkataraman J. Prevalence of nonalcoholic fatty liver disease and its biochemical predictors in patients with type 2 diabetes mellitus. E & C Hepatology. 2011; 7: 7-10.doi: 10.5604/17343038.969154
26. Chawla Y, Duseja A. Nonalcoholic steatohepatitis risk factors for significant liver disease . First consensus meet on NAFLD 2nd July 2005; 74.
27. Uchil D, Pipalia D, Chawla M, Patel R, Maniar S, Narayani, Juneja A. Non-alcoholic fatty liver disease (NAFLD)--the hepatic component of metabolic syndrome. J Assoc Physicians India. 2009 Mar;57:201-4.
28. Lee S, Jin KY, Yong JI, Hui KH. Obesity – The only independent factor in NAFLD. Scandinavian Journal of Gastroenterology. 2006; 41: 566-571.
29. Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M,et al. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. J Hepatol. 2004 May;40(5):781-6. doi: 10.1016/j.jhep.2004.01.028.
30. 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 Jun;37(6):1286-92. doi: 10.1053/ jhep. 2003.50 229.