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Non-Alcoholic Fatty Liver Disease: Evaluation of Serum Lipid Profile and Liver Enzymes in Different Grades of Sonographically Suggested Fatty Liver Subjects

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Aims: The aim of the study was to evaluate the fasting lipid profile & liver enzyme levels in different grades of sonographically suggested fatty liver subjects.

Methods& Results: This cross-sectional study included 155 subjects by non-random purposive sampling. For data analysis ANOVA, Chi square test were used and significance was defined by p value ≤0.05. In the present study, it was found that the total cholesterol was significantly increased (p= 0.007) with the increasing grades of fatty liver. Serum ALT and AST levels were also significantly increased with the increasing grades of fatty liver (p=0.001).

Conclusion: Ultrasonography along with serum fasting lipid profile & liver enzyme tests are essential for early detection of progression of non-alcoholic fatty liver disease (NAFLD).

Keywords: Non-alcoholic fatty liver disease, Ultrasonography, Alanine transaminase, Aspartate transaminase, Lipid profile.

Nonalcoholic fatty liver disease (NAFLD) is a worldwide epidemic which is mostly asymptomatic and progresses slowly to end stage liver disease (1). NAFLD involves a whole spectrum of liver pathologies from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis and hepatocellular carcinoma (2). It is now considered as a hepatic feature of metabolic syndrome, considered as rapidly growing disease in both developed and developing countries and is probably the most common cause of abnormal liver function tests worldwide. The prevalence of NAFLD has doubled during last 20 years, whereas the prevalence of other chronic liver diseases has remained stable or decreased (3).

Liver plays an important role in lipid metabolic pathways by taking up serum free fatty acid, manufacturing, storing and transporting lipid metabolites (4). The presence of dyslipidemia (hypercholesterolemia, hypertriglyceridemia or both) has been reported in 20 to 80% of cases associated with NAFLD (5). Chronic liver disease is often identified by asymptomatic elevations of two serum transaminases: alanine transaminase (ALT) and aspartate transaminase (AST) during routine serum biochemistry. Mild elevation of transaminase enzymes is a marker for significant liver disease (6).

Most individuals with NAFLD are asymptomatic and

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diagnosed by abdominal ultrasound performed for another reason. Liver biopsy is the most accurate method to diagnose a fatty liver. However, since it is invasive it is only used when the other noninvasive methods are inconclusive. Among the noninvasive methods, ultrasound is the preferred one because it is safe, non-invasive, non-radiation, widely available, cost effective and popular tool in the detection of fatty liver (7).

In a developing country like Bangladesh, fatty liver disease is rapidly increasing. So, this study was designed to evaluate the fasting lipid profile and liver enzyme levels in different grades of fatty liver subjects diagnosed by ultrasound.

PATIENTS & METHODS

This cross-sectional study was carried out at the Institute of Nuclear Medicine & Allied Sciences (INMAS), Dhaka Medical College Hospital Campus, Dhaka over a period of one year. Total 155 patients were included by non-random purposive sampling. Sonographically detected fatty liver subjects aged 18 years or more were included. But patients with positive hepatitis B and C virus surface antigen, liver cirrhosis, autoimmune hepatitis, history of alcohol consumption, any hepatotoxic drug intake, any chronic debilitating illness & pregnancy were excluded from the study.

At first clinical history, anthropometric measurements (standing height, weight, BMI) were recorded in a structured data collection sheet. Abdominal ultrasound scanning was performed in all participants by a skilled sonologist, who was blind to all clinical and laboratory data of patients, using Accuvix V20 scanner, Samsung Medison (South Korea) with a 3.5-MHz curvilinear transducer. US examination was performed after 6 hours fasting. Each subject was examined in the supine and left lateral positions during quiet inspiration. The presence of grading of fatty infiltration of the liver were recorded as: Grade 1: Slight, diffuse increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders (Figure- 1)

Grade 2: Moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm ((Figure-2).

Grade 3: Marked, diffuse increase in fine echoes with poor or non-visualization of the intrahepatic vessel borders, diaphragm and posterior right lobe of liver ((Figure- 3)

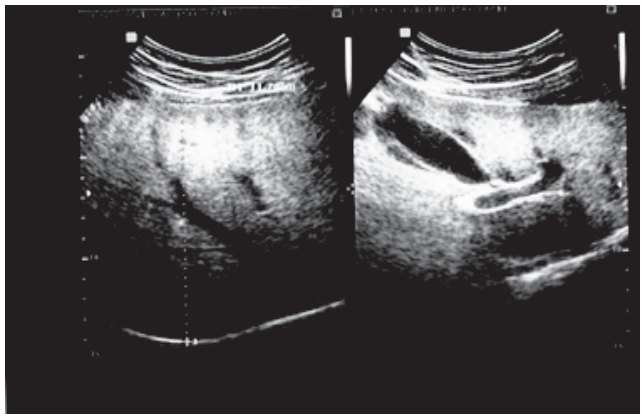

Figure-1: Ultrasound image scan showing slightly increased hepatic parenchymal echogenicity with well visualization of diaphragm and intrahepatic vessels borders (grade 1 fatty liver).

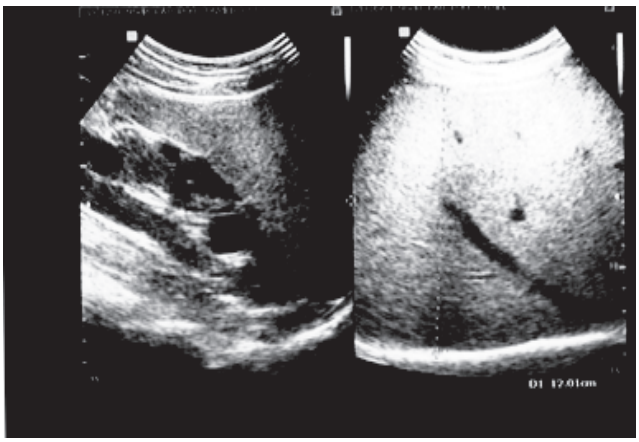

Figure-2: Ultrasound scan showing moderately increased hepatic parenchymal echogenicity with visualization of diaphragm and intrahepatic vessels borders (grade 2 fatty liver).

Blood sample was collected to measure fasting lipid profile (total Cholesterol, Triglyceride, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol) and Liver enzymes test (ALT, AST). Normal reference laboratory values for Cholesterol:140-200 mg/dl, HDL: >35 mg/dl, LDL: <140 mg/dl, TG: 40-160 mg/dl and ALT: <40 U/L, AST: <37 U/L.

Statistical Analysis:

Statistical analysis was performed using SPSS package 16.0 (SPSS Inc, Chicago, Illinois,

USA). Categorical data was expressed in percentage or number. Parametric data was expressed in mean ± SD. Parametric data was evaluated by ANOVA test. Categorical data was evaluated by Chi square test. Significance was defined by p value ≤0.05.

RESULTS

A total of 155 subjects with fatty liver were included in this study, out of them 82 (52.9%) were grade 1, 50 (32.3%) were grade 2 and 23 (14.8%) were grade 3 (Figure 1). Male to female ratio was almost 2:3 in the whole study subjects (Figure 2). The mean age was found 39±10.9 years in Grade 1, 39.5±10.4 years in Grade 2 and 41.4±7.1 years in Grade 3 (Table 1). The mean BMI was found 26.9±3.6 kg/m² in Grade 1, 29.2±4.5 kg/m² in Grade 2 and 30.0±4.1 kg/m² in Grade 3 (Table 2). Table 3 showed that total cholesterol was 166.55±47.55 mg/dl in Grade 1, 172.6±51.6 mg/dl in Grade 2 and 203.21±47.21 mg/dl in Grade 3, which were statistically significant (p=0.007). But other lipid profile parameters were not statistically significant. Table 4 showed that serum ALT and AST were significantly increased with the increasing grades of fatty liver (p=0.001).

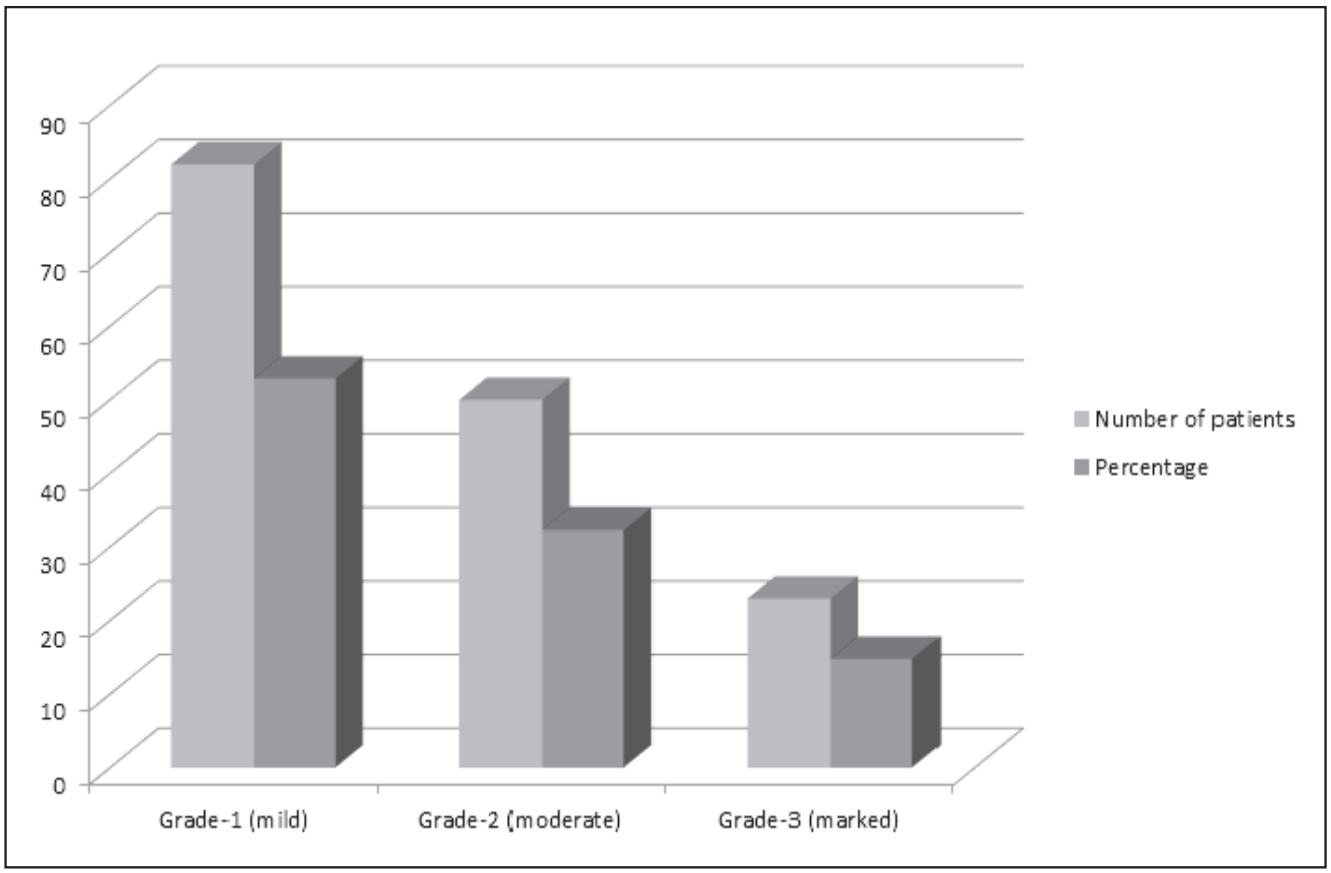

Figure-1: Distribution of the study population according to grades of fatty liver (n=155)

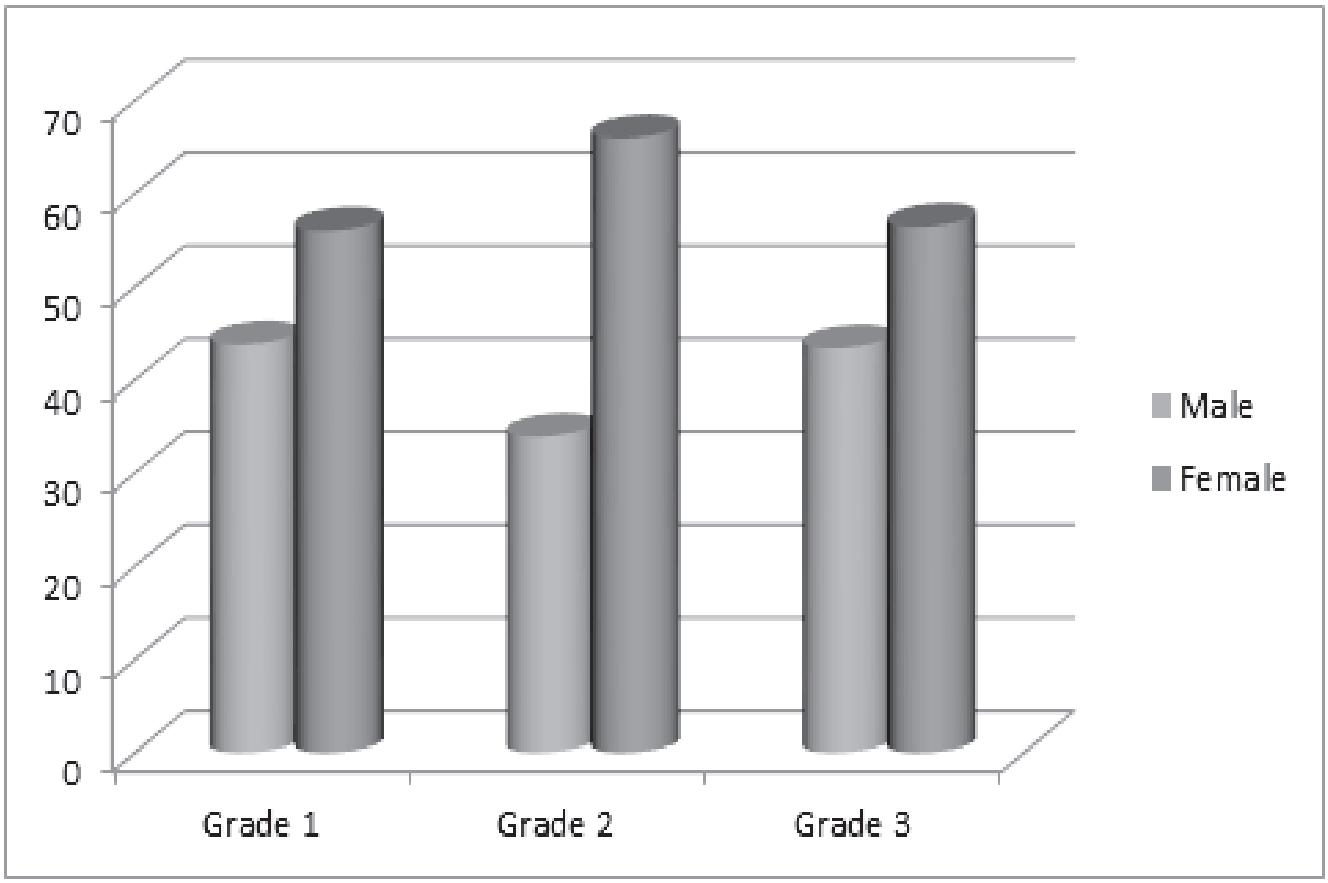

Figure-2: Distribution of the study population according to sex in different grades of fatty liver (n=155)

Table-1: Distribution of the study population by age in different grades of fatty liver (n=155)

Age (in year)	Fatty liver						P value
	Grade 1 (n=82)		Grade 2 (n=50)		Grade 3 (n=23)		
	n	%	n	%	n	%	
18-27	7	8.5	5	10.0	0	0.0	
28-37	35	42.7	19	38.0	4	17.4	
38-47	21	25.6	14	28.0	15	65.2	
48-57	14	17.1	9	18.0	4	17.4	
58-67	4	4.9	3	6.0	0	0.0	
68-77	1	1.2	0	0.0	0	0.0	
Mean±SD	39.0±10.9		39.5±10.4		41.4±7.1		0.613^{ns}
Range (min,max)	18,77		18,65		28,55		

Table-2: Distribution of the patients according to BMI in different grades of fatty liver (n=155)

BMI (kg/m^2)	Fatty liver						P value
	Grade 1 (n=82)		Grade 2 (n=50)		Grade 3 (n=23)		
	n	%	n	%	n	%	
<18.5 (Low)	1	1.2	0	0.0	0	0.0	
18.5-24.9 (Normal)	21	25.6	8	16.0	2	8.7	
≥ 25 (Over weight)	60	73.2	42	84.0	21	91.3	
Mean±SD	26.9±3.6		29.2±4.5		30.0±4.1		0.001^s
Range (min,max)	15.6,37.9		19,45		21.2,39		

Table-3: Distribution of the patients according to fasting lipid profile with grades of fatty liver (n=155)

Fasting lipid profile	Grade 1 (n=82)		Grade 2 (n=50)		Grade 3 (n=23)		P value
	Mean	±SD	Mean	±SD	Mean	±SD	
Total cholesterol (mg/dl)	166.55	±47.55	172.9	±51.6	203.21	±47.2	0.007^s
Range (min,max)	91.03	,293	88	,310	122	,314	
LDL-C (mg/dl)	105.9	±30.16	109.28	±31.0	120.92	±34.58	0.126^{ns}
Range (min,max)	11.5	,234	34	,184	80	,239	
HDL-C (mg/dl)	41.68	±8.36	43.59	±15.06	37.37	±8.06	0.081^{ns}
Range (min,max)	25	,65	28	,122.1	19.9	,59	
TG (mg/dl)	158.78	±53.12	175.83	±81.2	175.47	±61.26	0.268^{ns}
Range (min,max)	100	,401	99	,566	30.09	,313	

Table-4: Distribution of the patients according to liver enzymes status with grades of fatty liver (n=155)

Liver function test	Grade 1 (n=82)		Grade 2 (n=50)		Grade 3 (n=23)		P value
	Mean	±SD	Mean	±SD	Mean	±SD	
ALT (U/L)	36.55	±6.9	46.6	±20.6	65.53	±34.54	0.001^s
Range (min,max)	22.5	,59.1	28.9	,136	21.2	,157	
AST (U/L)	24.39	±5.87	28.81	±9.24	37.8	±21.6	0.001^s
Range (min,max)	12	,41	12	,68	17	,103	

DISCUSSION

NAFLD is mostly asymptomatic and does not have any special clinical sign. So, laboratory investigation is important for diagnosis as well as management of fatty liver disease and it may cause lot of complications if not controlled accordingly.

In this study, it was observed that out of 155 subjects 82 (52.9%) had grade 1,50 (32.3%) had grade 2 and 23 (14.8%) had grade 3 fatty liver (Figure 1), is in similar with the study in Malaysia which reported a lower percent of moderate NAFLD but a higher percentage of mild NAFLD (8). Male to female ratio was almost 2:3 in the whole study subject (Figure 2), which indicates that fatty liver is more common in female subject, which is similar with Chung et al. study (9), where they found 62.4% were female. There was no statistically significant difference (p=0.613) in grading of fatty liver with age. Although the mean age (41.4±7.1) was higher in Grade 3 in this study (Table 1).

Mean BMI was significantly ($p<0.05$) higher in grade 3 (30.0±4.1) followed by grade 2 (29.2±4.5) and grade 1(26.9±3.6) (Table 2). The mean BMI of the whole study in Kirovski et al. found 26.4 ± 5.0 kg/m² indicated a tendency to be slightly overweight (10). Cheah et al. also found that patients with NAFLD had a significantly higher BMI than those without NAFLD. (The mean BMI of respondents was 26.88 ± 4.59 kg/m² indicates a tendency for overweight (11).

Study showed, the mean total cholesterol and LDL-C were increased with the grades of fatty liver but only the mean total cholesterol was statistically significant (p=0.007). Serum TG (p=0.268) and HDL-C levels (p=0.081) did not differ statistically (Table 3). A study done by Mahaling et al. compared serum lipid abnormalities with different grades of sonographically detected non-alcoholic fatty liver subjects and they found that increasing grades of NAFLD were significantly associated with increasing levels of serum total cholesterol (p=0.001), LDL-C (p=0.000) and VLDL-C (P=0.003) and decreasing HDL-C (p=0.000) (12). They did not find any association between serum triglyceride level (p=0.05) with increasing grades of NAFLD. Kirovski et al. reported that HDL-C levels were significantly lower in the NAFLD group (47.1 ± 13.1 mg/dl vs. 60.7 ± 19.4 mg/dl, $p<0.05$), while serum levels of total cholesterol, LDL and VLDL-cholesterol and triglycerides did not significantly differ (10).

The pathogenesis of NAFLD has remained poorly understood. Differences in body fat distribution or antioxidant systems, possibly in the context of a genetic predisposition, may eseplaew the related factors. Retention of lipids within hepatocytes, mostly in the form of triglycerides, is a prerequisite for the development of non-alcoholic fatty liver disease. The primary metabolic abnormalities leading to lipid accumulation is not well understood, but they could consist of alteration in the pathways of uptake, synthesis, degradation or secretion in hepatic lipid metabolism resulting from insulin resistance. Insulin resistance is the most reproducible factor in the development of non-alcoholic fatty liver disease (13).

This study found that the mean ALT and AST were significantly ($p<0.05$) elevated with increasing grades of fatty liver (Table 4). Mazo et al. reported that higher serum AST and GGT levels were associated with the severity of NAFLD while serum levels of ALT did not significantly differ (14). On the other hand, Eshraghian et al. found that higher serum ALT and AST levels were associated with NAFLD (15). Another study done by Najeeb et al. also found that serum ALT & AST were significantly increased with the increasing grades of fatty liver (16).

Liver enzymes specially ALT plays a major role in gluconeogenesis, so ALT seems to be more related to the stored liver fat reserved than AST. Some authors reported that minor elevation of ALT levels may be a good predictor of mortality from liver disease. Elevation of ALT and AST levels are very common findings in NAFLD (17).

Study population was selected from one hospital of Dhaka city, so the results of the study may not reflect the exact picture of the country. Small sample size was also a limitation of the present study.

CONCLUSION

Serum total cholesterol, ALT and AST levels were significantly increased with the increasing grades of fatty liver detected by ultrasound. Thus, ultrasound along with serum fasting lipid profile and liver enzyme test are essential for early detection of progression of NAFLD.

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