

## Study of Lipid Profile in Different grades of Non- Alcoholic Fatty Liver Disease

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### ABSTRACT

**Background:** The prevalence of NAFLD (non- alcoholic fatty liver disease) is increasing as it exceed (31.79%) in Middle East and the metabolic syndrome prevalence in patients with NAFLD ranges from 18% to 67%, according to the body weight. Imaging or histology is very important tools in diagnosis of NAFLD. Early detection of NAFLD is very important especially if there is a non-invasive method. The clinicians in the primary, secondary and tertiary care are in need for specific and sensitive diagnostic tool that can be used easily.

**Aim:** To study and analyze serum lipid changes in non-alcoholic fatty liver disease of different grades.

**Patients and methods:** 100 patients diagnosed by ultrasonography as NAFLD, whose age was more than 18 years, were included in the study. History taking, full clinical examination and anthropometric measurement of weight, height and body mass index (BMI) were done for all patients. Serum lipid profiles including total cholesterol, HDL, LDL, triglycerides, ALT, AST. CBC, fasting and 2hpp blood glucose and TSH were measured.

**Results:** The largest group of patients (38%) was in the fifth decade of life. followed by 30% in the sixth decade of life. As the grade of NAFLD increased, there was associated significant increase in levels of serum total cholesterol (P-value 0.005), TG (P-value 0.002) LDL (P-value 0.001) and VLDL (P-value 0.003) and associated significant decrease in HDL (P-value 0.001).

**Conclusions:** abdominal ultrasonography and increased lipid profile values can be used to detect NAFLD early.

**Key words:** NAFLD, Lipid Profile, Ultrasonographic grading of NAFLD

### INTRODUCTION

NAFLD in developed countries is the most prevalent chronic liver disease. The Middle East has reported the highest prevalence of NAFLD (31.79%) and South America (30.45%) but Africa has the lowest prevalence rate (13.48%)<sup>(1)</sup>.

NAFLD develops due to abnormal triglycerides accumulation in the liver cells, leading to histologically benign disease named simple steatosis but it can progress to histologically aggressive form characterized by inflammation and liver cell injury with or without fibrosis called steatohepatitis (NASH)<sup>(2)</sup>.

Non-alcoholic fatty liver disease diagnosis needs (1) exclusion of history of heavy alcohol consumption and steatogenic drug use (2) detection of steatosis by histology or imaging<sup>(3)</sup>.

By definition, NAFLD is the presence of more than 5% hepatic steatosis in the absence of hepatocellular injury but in NASH there is more than 5% hepatic steatosis with presence of inflammation and hepatocellular injury with or without fibrosis<sup>(3)</sup>.

Visceral obesity, dyslipidemia, type 2 diabetes mellitus and metabolic syndrome are the most important risk factors for NAFLD. In lipid metabolism, thyroid hormones play an essential role. Hypercholesterolemia caused by decreased thyroid hormone levels has a fundamental role in the development of NAFLD<sup>(4)</sup>.

NAFLD and cardiovascular diseases share the similar risk factors. The mortality and morbidity of

cardiovascular disease are increased in the presence of NAFLD<sup>(5)</sup>.

Most NAFLD cases have a benign course without symptoms or signs of liver disease but some cases can be progressive and develop fibrosis and cirrhosis or even liver failure and hepatocellular carcinoma<sup>(6)</sup>.

Ultrasound can detect the liver fatty infiltration but cannot differentiate between NAFLD and NASH. This differentiation needs a liver biopsy and is essential because of the much higher risk of liver fibrosis and cirrhosis with NASH than NAFLD. However, liver biopsy is clinically impractical because of the large numbers of patients with NAFLD and that most patients are asymptomatic and its high cost and sampling errors<sup>(7)</sup>. Also it is a painful procedure with dangerous complication like bleeding<sup>(8)</sup>.

The ultrasound has moderate accuracy and is widely available so it is the primary tool to diagnose NAFLD in routine practice<sup>(9)</sup>.

### PATIENTS AND METHODS

This is a cross sectional prospective study. Total 100 patients older than 18 years were enrolled in this study. They were diagnosed by ultrasound as non-alcoholic fatty liver disease. An informed consent was taken from all patients. The American Gastroenterology Association criteria were used for classification of the patients<sup>(10)</sup>.

All the ultrasound examinations were performed on Voluson E6 US Scanners using 3-5 MHz.

*NAFLD Grades by ultrasound*

**Grade (I)** mild increase in the liver echogenicity. The diaphragm and intrahepatic blood vessel are normally visualized. **Grade (II)** moderate increase in echogenicity of the liver obscuring periportal echogenicity but the diaphragm is still visualized. **Grade (III)** severe increase in the liver echogenicity. Both the intrahepatic blood vessel and diaphragm are poorly visualized.

History taking and full clinical examination were done for all patients. The weight, height and body mass index (BMI) were measured for all participants. Overweight was calculated as BMI between 25 and 30 kg/m<sup>2</sup>. Obesity was calculated as BMI more than 30 kg/m<sup>2</sup>.

Serum triglycerides, total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein, ALT, AST, were measured for all patients and were compared in different grades of these patients. Complete blood picture, fasting and 2hpp blood glucose, TSH were done for all patients.

**Exclusion criteria:**

- 1- Patients with heavy alcohol intake (more than 20 gram per day).
- 2- Other diseases that may cause the liver condition such as chronic hepatitis C and B and HIV.
- 3- Hypothyroidism.

**Statistical methods**

Data were entered in the SPSS version 16 for windows and was analyzed. Frequency, Mean values, standard deviations were calculated for individual

variables. Analysis of different grade of NAFLD with variable component of lipid profile was done using analysis of variance (ANOVA) test. P value was calculated and value less than 0.05 was considered significant.

**RESULTS**

Our study was conducted on 100 patients diagnosed by ultrasound as non-alcoholic fatty liver disease. Out of them 58% were males and 42% were females.

**Table (1): Demographic data of the studied groups**

Age distribution	NO. of subjects (n= 100)	Percentage (%)
20-29	3	3%
30-39	19	19%
40-49	38	38%
50-59	30	30%
60 and above	10	10%
Total	100	100%

The largest proportion of our patients (38%) were between 40 and 49 years, 30% of our patients were between 50 and 59 years, 19% between 30 to 39 years, 10% were 60 year and above and 3% between 20 to 29 year.

The mean age of the participants ± SD was 47.25 ± 8.98. The Minimum age was 27 years and the maximum age was 66 years. The largest groups of patient were in the fifth and sixth decades.

**Table (2): Age distribution of the studied group**

Age group	Grade 1 (51)	Grade 2 (42)	Grade 3 (7)		P-value
20-29	3 (5.9)	0 (0.0)	0 (0.0)	Fischer exact= 38.33	0.001*
30-39	16 (31.4)	3 (7.1)	0 (0.0)		
40-49	24 (47.1)	14 (33.3)	0 (0.0)		
50-59	8 (15.7)	19 (45.2)	3 (42.9)		
60 and above	0 (0.0)	6 (14.3)	4 (57.1)		

Our study had showed statistically significant correlation of age with grades of NAFLD (p-value 0.001).

**Table (3): BMI classification among the studied groups**

Items	N=100	
<b>BMI in kg/m<sup>2</sup>:(Mean ±SD)</b>	<b>29.87 ± 4.63</b>	
<b>BMI Classification:</b>	<b>NO.</b>	<b>%</b>
Normal	19	19.0%
Overweight	31	31.0%
Obese	50	50.0%

BMI of the participants varied from 22.5 to 42.1 kg/m<sup>2</sup> with mean value of 29.87±4.63 kg/m<sup>2</sup>. 19% of the studied group had normal BMI, 31% were overweight and 50% were obese.

**Table (4): BMI classification in grades of NAFLD**

BMI classification			Grade			Chi-	p-value
			Grade 1	Grade 2	Grade 3		
Normal	Count		12	6	1	7.326	0.120
	% within grade		23.5%	14.3%	14.3%		
overweight	Count		14	12	5		
	% within grade		27.5%	28.6%	71.4%		
Obese	Count		25	24	1		
	% within grade		49.0%	57.1%	14.3%		
Total	Count		51	42	7		
	% within grade		100.0%	100.0%	100.0%		

In our study no association was found between grades of NAFLD and BMI P-value is 0.120.

**Table (5): Sex distribution among the studied groups**

			Grade			Chi-square	p-value
			Grade 1	Grade 2	Grade 3		
Sex	Male	Count	29	23	6	2.415	0.299
		% within grade	56.9%	54.8%	85.7%		
	Female	Count	22	19	1		
		% within grade	43.1%	45.2%	14.3%		
Total	Count	51	42	7			
	% within grade	100.0%	100.0%	100.0%			

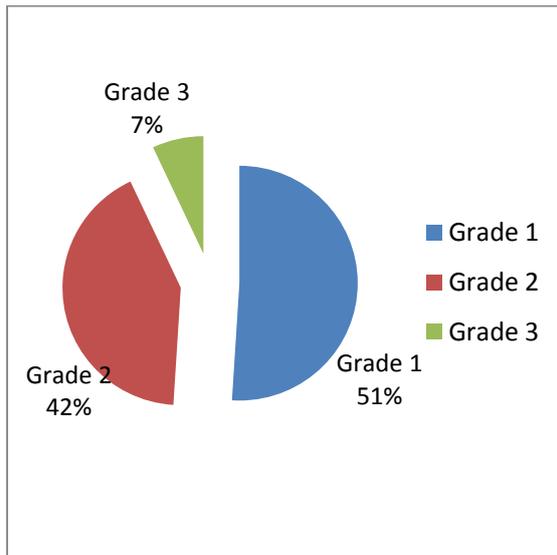
In our study no association was found between grades of NAFLD and sex P-values is 0.299.

**Table (6): DM and HTN classification among the studied groups**

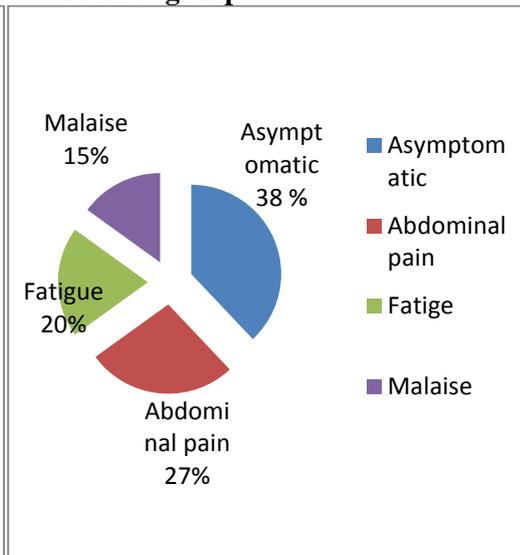
		D.M		Total
		Diabetic	not diabetic	
HTN	hypertensive	7	11	18
	not hypertensive	30	52	82
Total		37	63	100

Out of 100 subjects included in the study 37% were diabetic, 18% were hypertensive and 7% were both diabetic and hypertensive.

**Figure (5): Grades of NAFLD among the studied group**



**Figure (6): Symptoms classification among the studied group**



Out of 100 subjects included in the study 51% were grade I, 42% were grade II and 7% were grade III. Out of 100 subjects included in the study 38% were asymptomatic, 27% complained of abdominal pain or discomfort, 20% complained of fatigue and 15% complained of malaise.

**Table (9): liver enzymes in different grades of NAFLD**

Liver enzymes		Grade			Chi-square	p-value
		Grade 1	Grade 2	Grade 3		
<b>Normal</b>	Count	47	29	3	13.341	0.001
	% within grade	92.16%	69.05%	42.86%		
<b>Elevated</b>	Count	4	13	4		
	% within grade	7.84%	30.95%	57.14%		
<b>Total</b>	Count	51	42	7		
	% within grade	100.0%	100.0%	100.0%		

**Table (10): Comparison between NAFLD grades and the lipid profile**

Groups Items	Grade			Test of significance ANOVA	P value		
	Grade 1 (N=51)	Grade 2 (N=42)	Grade 3 (N=7)		G1-G2	G1-G3	G2-G3
<b>T. Cholesterol</b> in (mg/dl): (Mean ± SD)	202.55±46.49	232.4±44.16	233.0±33.41	F=5.579 p value=0.005	0.000*	0.008*	1.0
<b>TG</b> in (mg/dl) : (Mean ± SD)	168.98±46.03	202.57±59.4	226.71±50.66	F=6.888 p valu= 0.002	0.000*	0.001*	0.29
<b>HDL</b> (U/L ): (Mean ± SD)	50.17±8.73	44.43±8.72	39.85±5.98	F=7.795 p value=0.001	0.001*	0.002*	0.36
<b>LDL</b> (U/L ): (Mean ± SD)	125.15±27.65	148.78±31.29	146.71±31.1	F=7.852 p value 0.001	0.000*	0.056	1.0
<b>VLDL</b> (U/L ): (Mean ± SD)	28.49±7.54	34.83±11.01	35.85±9.61	F=6.133 0.003	0.000*	0.022*	1.0

The liver enzymes were elevated in 21% of the studied group. Our study had showed statistically significant correlation of enzyme elevation with grades of NAFLD (p-value 0.001).

The Analysis of Variance Test (ANOVA) was used to compare lipid changes in NAFLD grades and it was considered significant if the P values less than 0.05. It was noted that as the grade of NAFLD increased, there were associated significant increase in levels of serum total cholesterol (P-value 0.005), TG (P-value 0.002), LDL (P-value 0.001) and VLDL (P-value 0.003) and associated significant decrease in HDL (P-value 0.001).

Results of Bonferroni corrected post-hoc t-tests for each group of the three data sets. The Bonferroni corrected p-value is < 0.05 between grade1 and (grade2,3) for total cholesterol, TG, and HDL .

## DISCUSSION

Our study was conducted on 100 patients diagnosed by ultrasound as non-alcoholic fatty liver disease. The American Gastroenterology Association criteria were used for classification of the patients <sup>(10)</sup>.

In the present study males are predominant as compared with females; 58 males (58%) and 42 females (42%). In a study by Tanwani *et al.*, majority of participants 203 (67.7%) were males and females were 97 (32.3%) <sup>(11)</sup>. In another study by Bhusal *et al.*, males were 67% and females were 33% <sup>(10)</sup>.

The mean age of the participants  $\pm$  and SD was  $47.25 \pm 8.98$ . The minimum age was 27 years and the maximum age was 66 years. This is comparable to other study conducted by Sen *et al.* in which mean age of subjects were  $46.65 \pm 15.06$  years <sup>(12)</sup> and Mahaling *et al.* had shown mean age as  $49.14 \pm 9.65$  years <sup>(13)</sup>. The western communities' studies had shown mean age of subjects ranging from 41 - 45 years. In this study it is observed that our subjects were 5 - 10 years elder than the subjects studied elsewhere, this is because the largest groups of patient in our study were in the fifth and sixth decades.

Our study had showed statistically significant positive correlation of age with grades of NAFLD (p-value 0.000).The steatosis grade was found to be increased with the advancement of age. This agrees with the results of Frith *et al* and Mahmoud *et al* who reported that NAFLD prevalence increases with advancement of age. <sup>(14, 15)</sup>.

BMI of the participants varied from 22.5 to 42.1 kg/m<sup>2</sup> with mean value of  $29.87 \pm 4.63$  kg/m<sup>2</sup>. 19% of the studied group had normal BMI, 31% were overweight and 50% were obese. This is comparable to other study conducted by Sen *et al.* in which mean BMI of subjects were  $29.5 \pm 3.34$  kg/m<sup>2</sup> <sup>(12)</sup> and Bhusal *et al.* had shown mean BMI as  $27.97 \pm 4.28$  kg/m<sup>2</sup> <sup>(10)</sup>. In our study no association was found between grades of NAFLD and BMI and sex. This agrees with the results of Sen *et al* <sup>(12)</sup>.

Out of 100 subjects included in the study, grade I found to be the highest 51% followed by grade II 42% and grade III 7%.

During the diagnosis, the majority of NAFLD patients were asymptomatic and the disease was discovered incidentally. 38% of the patients in this study were a symptomatic and this is comparable to Indian studies but much less than Western studies that have reported asymptomatic patients between 47.7 % and 64%.

Visceral obesity, dyslipidemia, type 2 diabetes mellitus and metabolic syndrome are the most important risk factors for NAFLD. Out of 100 patients in this study 37% had diabetes mellitus, 18% were hypertensive and 7% were both diabetic and hypertensive.

We have observed alteration in LFT in the patients with NAFLD and the parameters which were altered are SGOT (AST) in 21% of patients and SGPT (ALT) in 17% of patients. This is consistent with the results obtained by Hegazy *et al.* who reported that patients with NAFLD had higher levels of ALT than subjects with healthy liver <sup>(16)</sup>. We have also observed that as the grade of NAFLD increased, there were associated significant enzyme elevations, increasing the probability of steatohepatitis.

The total cholesterol was elevated in 58% of the studied group (20% is borderline high and 38% is high). TG was elevated in 61% of the studied group (25% is borderline high and 36% is high). HDL decreased in 49% of the studied group. LDL was elevated in 49% of the studied group (20% is borderline high and 13% is high and 16% is very high). VLDL was elevated in 39% of the studied group. This is comparable to other study by Mahaling *et al.* which reported that the total cholesterol, TG, LDL and VLDL were elevated in 45.71%, 67.14%, 34.28%, and 25.71% respectively and HDL decreased in 62.85% <sup>(13)</sup> and Tanwani *et al.* in which total cholesterol, TG, LDL and VLDL were elevated in 62%, 50.7%, 39%, 39% respectively and HDL decreased in 51.7% <sup>(11)</sup>.

Dyslipidemia was observed in 91% of participants. The mean value of the total cholesterol, TG, LDL, VLDL, HDL were  $217.22 \pm 46.83$ ,  $187.13 \pm 55.39$ ,  $136.59 \pm 31.43$ ,  $31.67 \pm 9.76$ ,  $47.04 \pm 9.15$  respectively.

It was noted that as the grade of NAFLD increased, there were associated significant increase in levels of serum total cholesterol (P-value 0.005), TG (P-value 0.002), LDL (P-value 0.001) and VLDL (P-value 0.003) and associated significant decrease in HDL (P-value 0.001). This agrees with the results of *Tanwani et al., Sen et al., Mahaling et al., and*

**Mahmoud et al.**<sup>(11,12,13,15)</sup> and disagrees with **Bhusal et al.**<sup>(10)</sup> because of the inability of **Bhusal et al.** to enroll participants from severe NAFLD category.

To summarize, ultrasound can be used as a primary tool for diagnosis of NAFLD. It is available and relatively inexpensive method that can detect various grades of NAFLD supported by increased lipid profile values.

Liver biopsy is the most accurate in diagnosis of NAFLD But it is clinically impractical because of the large numbers of patients with NAFLD and the high cost of liver biopsy and sampling errors<sup>(7)</sup>. Also it is a painful procedure with dangerous complication like bleeding<sup>(8)</sup>.

## CONCLUSION

Dyslipidemia was found in most patients of NAFLD so both abdominal ultrasonography and increased lipid profile values can be used to detect NAFLD early.

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