

Original Article

Comparison of Blood Biochemical Markers and Anthropometric Parameters in Different Fatty Liver Grades

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ABSTRACT

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Keywords

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Background and Aims: Obesity and hyperlipidemia, diabetes, and malnutrition are among the causes of fatty liver disease. This study compares blood biochemical markers and anthropometric parameters in different grades of fatty liver.

Materials and Methods: In this descriptive cross-sectional study, 73 fatty liver patients were studied. The degree of fatty liver disease was divided into three grades on ultrasonography. Anthropometric parameters BMI, waist circumference, height, weight in the fasting state were evaluated according to the standard protocols. The blood samples were taken and biochemical variables fasting blood sugar, serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic-pyruvic transaminase (SGPT), calcium, phosphorus, low density lipoprotein (LDL), cholesterol, triglyceride, and others were examined with photometric and HPLC methods.

Results: Statistical analysis was significant between grades 1 and 3 of fatty liver ($p = 0.006$) and body mass index between grades 1 and 3 of fatty liver ($p = 0.003$). Comparing SGOT between different grades did not show any significant differences. However, comparing Bili Total indicated a significant difference between grades 1 and 2 and 1 and 3. Moreover, statistical analysis of qualitative variables, such as gender, hypertension, smoking, drug, alcohol, heart disease, stomach disease, and kidney disease, was not statistically significant between the three fatty liver groups. Median \pm IQR had a significant difference for SGOT and Bili Total ($p < 0.05$).

Conclusion: This study showed the significance of BMI, waist circumference, and bilirubin factors in different grades of fatty liver. Monitoring BMI, waist circumference, and bilirubin factors will be useful for susceptible people to non-alcoholic fatty liver disease.

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Introduction

The fat existence in the liver is normal, but the fatty liver disease occurs if the amount of fat is more than 5 to 10% of the liver's total weight. There are no specific symptoms of the disease, but it can cause indigestion if it progresses in the liver and is not being cared for. Fat deposition in the liver is called fatty liver. Alcohol consumption can cause this disease. Non-alcoholic fatty liver disease occurs when the liver has difficulty breaking down fats, and as fat deposits in the liver tissue, a person develops fatty liver. The types of the disease are not related to alcohol consumption and are determined when 10% or more of the liver's weight is fat. The disease itself is divided into several categories, and in the most severe stages, it causes liver cirrhosis or ulcers and then liver failure [1]. Although, non-alcoholic fatty liver may also have various other reasons, including obesity, fatty and fast foods, lack of physical activity, and eating inappropriate foods. However, consuming some drugs which deposit in the liver can also be considered a reason. Fatty liver is classified into four grades in terms of progression. Grade 1 fatty liver is the simplest type and is due to eating unhealthy foods. This grade can be eliminated by increasing physical activities and healthy eating. In the grade 2 fatty liver, the patient's condition becomes a little harder and more complicated. This condition of the fatty liver also has its diet. Grade 3 fatty liver can only be cured by improving lifestyle, including exercise, reducing stress, proper nutrition (reducing meat, fast foods, fats, and fatty

foods). It should be noted that this is a warning for people with fatty liver of grades 2 and 1 who are negligent in treating their disease. Finally, in grade 4 fatty liver, known as hepatic cirrhosis, a liver transplant is required if no action is taken to heal the disease. Disease staging and noninvasive assessment are based on sex, age, platelet count, lipid profile, body mass index (BMI), and liver function. These clinical parameters also can be used as prediction and prognosis factors in fatty liver disease [2, 3]. Moreover, imaging modalities, such as ultrasonography, transient elastography (TE), and magnetic resonance imaging mass spectroscopy, can be used for this purpose [4]. The risk of fatty liver is higher in persons with higher BMI (approximately 4.1 to 14-fold [5, 6]. In terms of gender, non-alcoholic fatty liver disease (NAFLD) is more common in men and postmenopausal women than premenopausal women, mainly caused by increased visceral fat accumulation [7]. Fatty liver can also occur in children over ten years of age. It is a chronic liver disorder and will last for years. The main cause of fatty liver is unknown, but several specific clinical disorders have been identified as underlying causes. Seventy percent of patients with fatty liver are obese [8]. Diabetes and high blood fats are also underlying causes. Various factors that alter the body's metabolism, including sudden weight loss, malnutrition, venous malnutrition, and prolonged hunger, may also cause fatty liver. A wide range of diseases such as high cholesterol, high blood triglyceride levels, metabolic

syndrome, obesity (especially when fat is concentrated in the abdomen), polycystic ovary syndrome, apnea, type 2 diabetes, hypothyroidism, and hypopituitarism increases the risk of non-alcoholic fatty liver disease [9]. Fatty liver is usually detected during routine clinical or laboratory checkups. Bilirubin, a sign of liver cell function, is normal in the early stages of the disease, but it raises the possibility of cirrhosis. Other laboratory indicators of liver function are normal in the early stages of fatty liver and are impaired only in the advanced stages (cirrhosis). Hyperlipidemia is observed as an increase in triglycerides, cholesterol, and especially low-density lipoprotein (LDL) cholesterol, as well as hyperglycemia in fatty liver disease, all of which are due to the presence of metabolic syndrome [10, 11]. Diabetes and high blood fats are also underlying causes. Various factors that change the body's metabolism, including sudden weight loss, malnutrition, venous malnutrition, and prolonged hunger, may also cause fatty liver. A wide range of diseases such as high cholesterol, high blood triglyceride levels, metabolic syndrome, obesity (especially when fat is concentrated in the abdomen), polycystic ovary syndrome, apnea, type 2 diabetes, hypothyroidism, and hypopituitarism increases the risk of non-alcoholic fatty liver disease [11]. In systematic studies, liver enzymes are elevated, and if the disorder is suspected, further action is needed to discover the underlying cause and investigate other causes of liver disease. Tests are necessary for blood sugar and lipids, as well as for hepatitis viruses. Imaging studies such as ultrasound show the

accumulation of fat in the liver. The liver enzymes as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are present in the liver cell and enter the patient's serum by destroying the liver cell. Their increase is a sign of liver cell destruction. Increased liver enzymes were not directly related to the severity of the disease and were observed in 50% of patients with fatty liver. This increase reaches 80% in the advanced stages of the disease. The increase in the above liver enzymes is between 1.5 to 2 times normal in most cases. Very high liver enzymes (more than 10 times the normal serum level) in fatty liver disease are very rare and raise the possibility of other liver diseases. Bilirubin is a waste product of blood made by the liver from the destruction of old red blood cells, and after detoxification, the liver is excreted in bile and urine. Increased bilirubin is an indicator of liver disease [10]. In this study, different blood parameters, height, and weight between people with three different grades of fatty liver were studied and the average for each parameter, for example, average BMI in people with grade 1 was calculated and compared with the average BMI of people with grades 2 and 3.

Materials and Methods

The case study criteria included the diagnosis of fatty liver by ultrasound under the supervision of an audiologist and confirmation of the diagnosis by a gastroenterologist. The persons with other types of liver disease such as hepatitis B or C and autoimmune hepatitis, a history of liver transplantation, kidney failure, following a diet to lose weight or taking pills

for weight loss for the past six months, and those using steroids, tamoxifen, pyroxylin, methotrexate, various statin drugs, supplementation of vitamins E or D in three months before of study and pregnant or lactating women were excluded from this study. After obtaining conscious consent, the present study was performed on 73 patients with different fatty liver grades referred to Isfahan's Salamat-Ara Clinic. Inclusion criteria included patients who were 25 to 65 years old who provided informed consent. Those developing other autoimmune liver diseases such as hepatitis B and C or history of liver transplantation and renal defect were excluded from the study. This study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Iran (IR.SSU.REC.117118) and the IRCT administration team, Iran University Campus (IRCT2012091310826N1). History of cardiovascular disease, infection, diabetes, gastric disease, high blood pressure, fat, smoking, and alcohol consumption was examined. An expert radiologist performed hepatic ultrasonography using an Aloka Alpha6 (Tokyo, Japan). It was performed with the same equipment at the first and end of the study. The research subjects who participated in this study should be fasting for eight hours. The presence or absence of cystic or solid tumors and calcification were studied during the liver ultrasonography. Also, the degree of fatty liver disease was divided into three levels on ultrasonography. Despite some limitations of ultrasonography for grading fatty liver disease, it

is a non-aggressive way for fatty liver disease screening due to availability.

Anthropometric measurements

Stadiometer (Seca) was used to measure the height, and a digital scale (Omron BF, Japan) was used to measure the participants' weight as standard protocols. The weight (kg) was divided by the height (m) square to calculate the BMI. Besides, waist circumference was measured from the midpoint of the lowest rib and iliac crest. Then, a mercurial sphygmomanometer (Seca) was used to measure blood pressure. All subjects were examined after an overnight fast. At the beginning of the research, the fasting blood samples were taken. The CommercialKit (Pars Azmoon, Tehran, Iran) was used to measure the serum calcium. High performance liquid chromatographic (HPLC) method was used to assay the concentration of 25(OH)-vitamin D with the USA Agilent system. AST and ALT levels were measured by the enzymatic photometric method with a sensitivity of U/L. Triglycerides (TGs) were measured through enzymatic methods, and commercially available enzymatic reagents (Pars Azmoon, Tehran, Iran). In addition, a biotechnical auto-analyzer was used to measure the alkaline phosphatase (ALP).

Statistical analysis

SPSS software (version 18) was used, and data are represented as the mean±SD. Statistical comparisons between groups were performed using the ANOVA test for the normal variables and Kruskal-Wallis and Bonferroni test for the variables that were not normal. In qualitative variables, three groups were compared with chi-square. P-value < 0.05 was considered significant.

Results

Items with a normal distribution ($p < 0.05$), including waist circumference and BMI variables, were subjected to Post-hoc statistical analysis. After performing Post-hoc for waist circumference and BMI, it was found that the Post-hoc test results for waist circumference between grade 1 and 3 fatty liver were significant ($p = 0.006$). Moreover, the Post-hoc test results for BMI between grade 1 and 3 fatty liver were significant ($p = 0.003$) (Table 1). Descriptive information was obtained, and Median \pm IQR was implemented instead of Mean \pm SD (Table 2) for variables that did not have a normal distribution ($p < 0.05$). Median \pm IQR had a significant difference for serum glutamic-oxaloacetic transaminase (SGOT) and Bili Total ($p < 0.05$). The comparison of Bonferroni adjustment for SGOT and Bili Total between grades 1 and 2, 1 and 3, 2 and 3 showed that different grades did not significantly differ. Also, in qualitative variables, three groups were compared with chi-square statistical analysis in Table 3.

Discussion

This study aimed to compare blood biochemical markers and anthropometric parameters in different grades of fatty liver. In a study done by Alavian et al. [12], the relationship between biochemical and anthropometric measures with NAFLD was investigated for school-aged children and adolescents in Iran. They concluded that NAFLD was significantly associated with increasing age, ALT, total cholesterol, LDL-

cholesterol, and triglyceride. Also, Lankarani et al. [13] did a population base study about the NAFLD in southern Iran. They reported that patients with NAFLD had a higher prevalence of hypertension, high fasting blood sugar (FBS), high cholesterol, high triglyceride, high waist circumference, and high BMI. Similar investigations have been conducted to study the relation of anthropometric and biochemical measures with fatty liver disease in some regions, including Eastern India, Seoul, Korea, Bangladesh, and others [14-18]. At first, in this study, NPar test statistical analysis was performed for all variables [19]. According to the results obtained from the NPar test, One-way analysis was performed for items that had a normal distribution, including (BMI, waist circumference, Chol, Age, SGPT, alkaline, 25 (OH) Vit D3, calcium and phosphorus, high density lipoprotein). Post-hoc statistical analysis was performed for the items with a normal distribution ($p < 0.05$), including waist circumference and BMI. After performing Post-hoc for waist circumference and BMI, we concluded that Post-hoc test results for waist circumference between grades 1 and 3 of fatty liver were significant ($p = 0.006$), and Post-hoc test results for BMI between grades 1 and 3 of fatty liver were significant ($p = 0.003$). A similar study had been conducted by Cutillas-Marco et al. [20], indicating that biochemical markers and lipid profiles are associated with NAFLD [21, 22]. Some references recommended for NAFLD prediction use anthropometric indicators such as BMI and waist circumference [23].

Table 1. One-way statistical analysis for the variables with normal distribution

Variables	No.	Mean	95% Confidence interval for mean		P-value
			Lower bound	Upperbound	
Body mass index (kg/m²)					
1.0*	8	27.28375 ± 1.697005	25.86502	28.70248	0.003
2.0	42	29.44800 ± 2.560973	28.64994	30.24606	
3.0	23	30.85000 ± 2.662786	29.69853	32.00147	
Total	73	29.65255 ± 2.699476	29.02271	30.28238	
Waist circumference (cm)					
1.0	8	100.250 ± 4.7434	96.284	104.216	0.007
2.0	42	104.643 ± 4.9869	103.089	106.197	
3.0	23	107.565 ± 6.8346	104.610	110.521	
Total	73	105.082 ± 5.9413	103.696	106.468	
Serum glutamic-pyruvic transaminase (IU)					
1.0	8	73.375 ± 25.9612	51.671	95.079	0.266
2.0	42	75.500 ± 24.0358	68.010	82.990	
3.0	23	85.348 ± 25.9118	74.143	96.553	
Total	73	78.370 ± 24.9558	72.547	84.192	
Alkaline Phosphatase (IU)					
1.0	8	232.500 ± 55.3508	186.226	278.774	0.718
2.0	42	218.000 ± 52.4916	201.642	234.358	
3.0	23	223.478 ± 37.2081	207.388	239.568	
Total	73	221.315 ± 48.0803	210.097	232.533	
25(OH)Vitamin D3(ng/ml)					
1.0	8	13.750 ± 4.3671	10.099	17.401	0.321
2.0	42	16.224 ± 4.8970	14.698	17.750	
3.0	23	15.057 ± 4.3421	13.179	16.934	
Total	73	15.585 ± 4.6874	14.491	16.679	
ca* P					
1.0	8	32.4563 ± 6.08334	27.3705	37.5420	0.701
2.0	42	31.8298 ± 6.69646	29.7430	33.9165	
3.0	23	33.1930 ± 5.35028	30.8794	35.5067	
Total	73	32.3279 ± 6.18592	30.8847	33.7712	
High-density lipoprotein (mg/dl)					
1.0	8	43.625 ± 11.5008	34.010	53.240	0.490
2.0	42	40.310 ± 9.5725	37.327	43.293	
3.0	23	39.209 ± 6.5659	36.369	42.048	
Total	73	40.326 ± 8.9342	38.242	42.411	
Cholesterol (mg/dl)					
1.0	8	195.000 ± 50.2679	152.975	237.025	0.595
2.0	42	209.595 ± 38.0904	197.725	221.465	
3.0	23	204.826 ± 33.6204	190.288	219.365	
Total	73	206.493 ± 37.9265	197.644	215.342	
Age					
1.0	8	48.3750 ± 11.95154	38.3833	58.3667	0.669
2.0	42	51.3095 ± 10.40049	48.0685	54.5505	
3.0	23	52.0435 ± 8.37463	48.4220	55.6649	
Total	73	51.2192 ± 9.90124	48.9090	53.5293	

*1.0: level one fatty liver; 2.0: level two fatty liver; 3.0: level three fatty liver

Table 2. Statistical analysis to calculate the Median \pm IQR and to evaluate the significance of these variables for the variables that did not have a normal distribution and P-value > 0.05

Variables	Median \pm IQR	P-value
Systolic blood pressure (mmHg)		
1	120.000 \pm 5.0	0.629
2	125.000 \pm 10.0	
3	130.000 \pm 20.0	
Diastolic blood pressure (mmHg)		
1	80.000 \pm 8.8	0.946
2	82.500 \pm 10.0	
3	85.000 \pm 10.0	
serum glutamic-oxaloacetic transaminase (IU)		
1	39.000 \pm 15.8	0.007
2	43.500 \pm 19.5	
3	63.000 \pm 31.0	
Bilirubin total (mg/dl)		
1	0.700 \pm 0.2	0.049
2	0.900 \pm 0.4	
3	1.000 \pm 0.2	
Bilirubin direct (mg/dl)		
1	0.3000 \pm 0.15	0.463
2	0.3000 \pm 0.16	
3	0.3000 \pm 0.10	
Calcium (mg/dl)		
1	9.350 \pm 0.9	0.723
2	9.250 \pm 0.5	
3	9.100 \pm 0.6	
Phosphorus (mg/dl)		
1	3.650 \pm 1.2	0.517
2	3.450 \pm 1.1	
3	3.800 \pm 1.4	
Fasting blood sugar (mg/dl)		
1	89.000 \pm 11.8	0.098
2	98.000 \pm 15.5	
3	97.000 \pm 16.0	
Low density lipoprotein (mg/dl)		
1	132.500 \pm 53.0	0.988
2	132.500 \pm 64.5	
3	116.000 \pm 56.0	
Cholesterol (mg/dl)		
1	208.000 \pm 90.3	0.285
2	225.500 \pm	
3	190.5	
	253.000 \pm 156.0	

Table 3. Statistical analysis of qualitative variables, which is investigated with chi-square

Qualitative Variables	Sono.Grade (1)			No. (%)	P-value
	1.0	2.0	3.0		
	N= 8	N= 42	N= 23		
Gender	Female	4 (50.0)	7 (16.7)	7 (30.4)	0.099
	Male	4 (50.0)	35 (83.3)	16 (69.6)	
Diabetes	No diabetes	8 (100.0)	35 (83.3)	18 (78.3)	0.360
	Diabetes	0 (0.0)	7 (16.7)	5 (21.7)	
Hypertension	No Hypertension	6 (75.0)	29 (69.0)	12 (52.2)	0.319
	Hypertension	2 (25.0)	13 (31.0)	11 (47.8)	
Triglyceride	No high Triglycerides	5 (62.5)	23 (54.8)	10 (43.5)	0.562
	High Triglycerides	3 (37.5)	19 (45.2)	13 (56.5)	
Cholestrol	No High Cholesterol	8 (100.0)	35 (83.3)	21 (91.3)	0.344
	High cholesterol	0 (0.0)	7 (16.7)	2 (8.7)	
Coronary artery disease	No Heart disease	8 (100.0)	41 (97.6)	22 (95.7)	0.791
	Heart disease	0 (0.0)	1 (2.4)	1 (4.3)	
Gastrointestinal disease	No Stomach disease	8 (100.0)	41 (97.6)	23 (100.0)	0.688
	Stomach disease	0 (0.0)	1 (2.4)	0 (0.0)	
Cigarette	Non-smoker	7 (87.5)	33 (78.6)	20 (87.0)	0.642
	Smoker	1 (12.5)	9 (21.4)	3 (13.0)	
Drug abuse	Non-drug user	8 (100.0)	40 (95.2)	23 (100.0)	0.468
	Drug user	0 (0.0)	2 (4.8)	0 (0.0)	

Interestingly, WC increases were also related to the risk of developing diabetes in subjects with prediabetes and NAFLD [24]. Mean FBS, glycosylated hemoglobin (HbA1c), fasting insulin were significantly higher among the lean NAFLD group than the obese NAFLD group. The obese NAFLD group had significantly higher SGPT and SGOT levels than the lean NAFLD group [25]. For variables that did not have a normal distribution ($p > 0.05$), first, descriptive information was obtained, and Median \pm IQR was implemented. Median \pm IQR had a significant difference for SGOT ($p < 0.05$) and a difference close to significant for Bili Total. Therefore, the comparison of Bonferroni adjustment for

SGOT and Bili Total between grades 1 and 2, 1 and 3, 2 and 3 of fatty liver was performed. The analysis results showed that the comparison of SGOT between different grades did not have a significant difference. Comparing Bili Total between grades 1 and 2 with $p = 0.071$ and between grades 1 and 3 with $p = 0.052$ had a difference close to significant. Also, for qualitative variables, the three groups were compared with chi-square. Also, statistical comparison of chi-square between three grades of fatty liver in patients with diabetes, hypertension, triglycerides, and high cholesterol, cardiovascular patients, and smokers did not show a significant difference compared to healthy (control) individuals. To

support this investigation and better understand the role of biochemical analytes as the risk factor of fatty liver disease, large sample size and more research are needed.

Conclusion

Abnormal metabolic variables and NAFLD had a strong relationship in adults. For the persons susceptible to NAFLD, it would be useful to monitor BMI, waist circumference, and bilirubin factors in different grades of fatty liver.

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Moreover, statistical analysis of comparison of qualitative variables, such as gender, hypertension, smoking, drug, alcohol, heart disease, stomach disease, and kidney disease between three groups of fatty liver was not statistically significant.

Conflicts of Interest

The authors stated that there is no conflict of interest regarding the publication of this article.

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