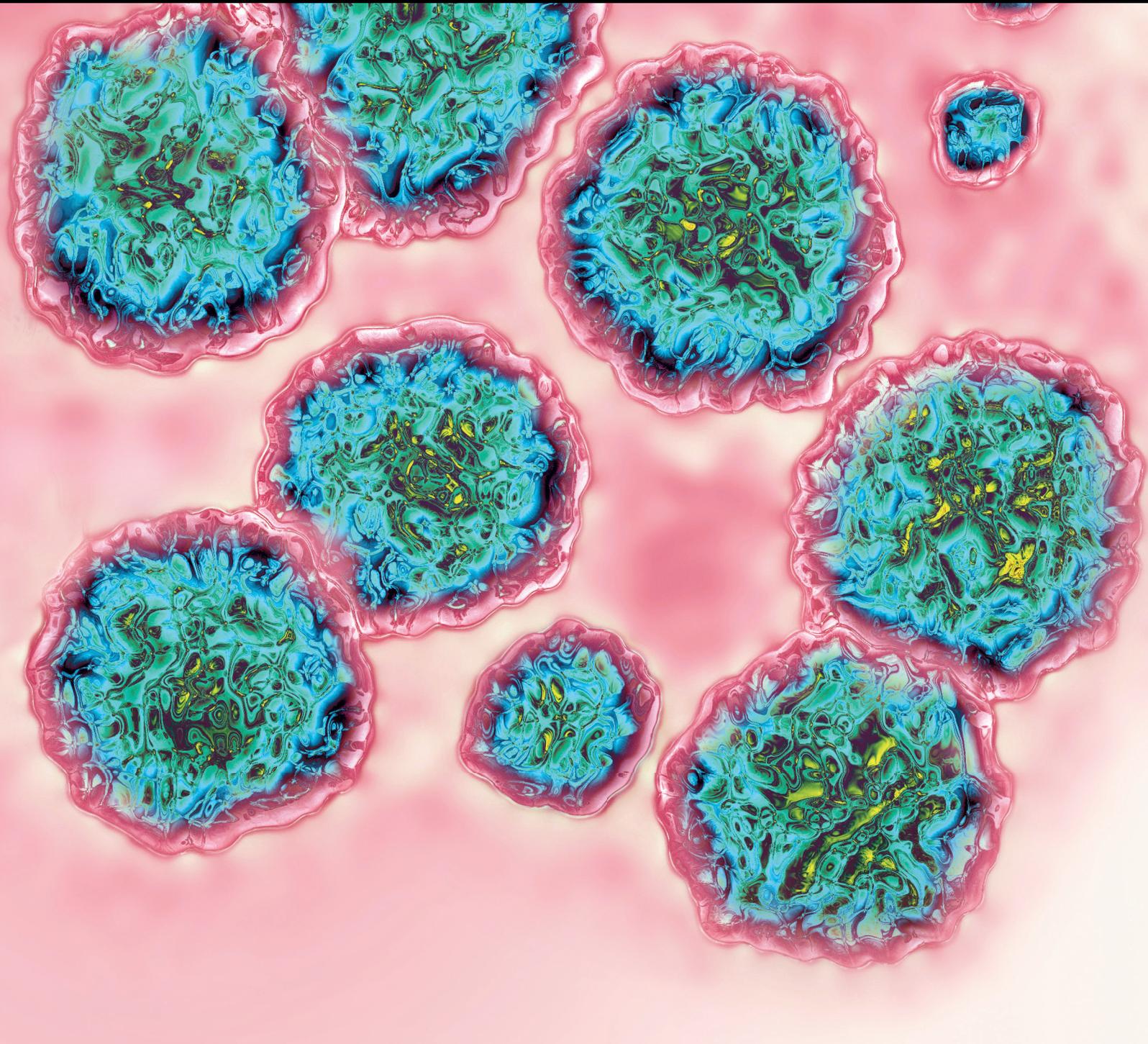


Nonalcoholic Fatty Liver Disease

Lead Guest Editor: Branka Filipović

Guest Editors: Alastair Forbes, Bojan Tepeš, and Dan Dumitrascu





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Canadian Journal of Gastroenterology and Hepatology

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Editorial

Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is regarded as the most significant liver disease from the twenty-first century in the Western world. Although its development is surely driven by environmental factors, it is also regulated by genetic background. NAFLD ranges over a wide spectrum, extending from nonalcoholic fatty liver (NAFL) which is generally benign, through to nonalcoholic steatohepatitis (NASH) to liver cirrhosis, end-stage liver disease, and even hepatocellular carcinoma (HCC) despite the absence of significant alcohol consumption. The relationship of NAFLD with metabolic alterations such as type 2 diabetes is well described and related to insulin resistance, with NAFLD being recognized as the hepatic manifestation of metabolic syndrome. However, NAFLD may also coincide with endocrine diseases such as polycystic ovary syndrome, hypothyroidism, growth hormone deficiency, or hypercortisolism. It is therefore essential to remember, when discovering altered liver enzymes or hepatic steatosis on radiological exams, that endocrine diseases can cause NAFLD. In the latest years, obstructive sleep apnea syndrome (OSAS) has been associated NAFLD. Experimental evidence suggests that chronic intermittent hypoxia may per whole trigger liver injury, inflammation, and fibrogenesis, and, interestingly, OSAS is also believed to be one of the elements promoting the evolution of NAFLD from steatosis to nonalcoholic steatohepatitis (NASH) [1–4].

Z. Prokopowicz et al. are stressing the importance of the early diagnosis of NONAL in obese children and NAFLD predictive risk factors include increased waist circumference, elevated waist-hip ratio and waist-to-height ratio, and elevated total cholesterol, triglycerides, and fasting insulin as

well as elevated glucose and insulin concentration in the OGTT and HOMA-IR index. NAFLD increases the risk of potential cardiovascular complications expressed by diagnosis of metabolic syndrome. The best independent predictive risk factor for diagnosing NAFLD in obese children is fasting insulin > 18.9 uIU/ml.

Shidfar et al. in their study aimed to examine the effect of virgin olive oil on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the severity of steatosis in the NAFLD patients undergoing a weight-loss diet. Definitive conclusion is that the consumption of a low calorie diet enriched with olive oil, along with slight weight reduction, reinforces the desired effects of weight loss in improving the levels of the hepatic enzymes.

Y. Zhai et al. in their present paper investigated the association between NAFLD and sarcopenia in elderly patients and concluded that NAFLD is not independently associated with sarcopenia in older patients.

Miyata and Miyata studied the correlation between the period from the first to each examination date and the mean value of FIB4 index during the past year to each examination date was analyzed. In their conclusion, they claimed that correlation estimated was thought to be the time-dependent change of the mean FIB4 index during the past one year and in the present study the correlation was proved to be extremely strong. The time-dependent change of FIB4 index and its increase-decrease rate per year could be approximately speculated.

Our team (B. Filipović et al.) has been involved for a while in cognitive changes exploration among different

gastroenterology patients. We hypothesized that NAFLD, as a condition, affects the brain tissue and, subsequently, the cognitive state. according to our results, patients with NAFLD had a greater risk to suffer from the cognitive impairment and depression: RR = 3.9; 95% CI 1.815–8.381; and RR = 1.65; 95% CI 1.16–2.36; .Briefly, NAFLD significantly influenced the cognitive deficit and tissue volume reduction and patients suffering from NAFLD had about four times higher risk of having a cognitive impairment.

T. Milovanovic et al. investigated whether platelet count (PC), mean platelet volume (MPV), and platelet distribution width (PDW) can predict the presence of liver fibrosis in this group of patients. In their conclusion, patients with NAFLD have significantly higher values of PCT, PDW, and MPV when compared to the healthy controls. Further studies are needed to establish their potential use for prediction of the degree of liver steatosis and fibrosis in NAFLD patients.

V. Nguyen et al. have analyzed clinical, anthropometric, and biochemical changes at six months and after consecutive treatment with two and three serials of intragastric balloons (IGB). They concluded that IGB therapy is an effective, alternative nonsurgical means for weight loss in the management of obesity and NAFLD over the short term, with greatest outcomes observed after six months. Improvements in insulin resistance and hepatic transaminases correlated with weight change.

Apparently, according to the papers published in this special issue, NAFLD is a serious problem, which each author from their own aspect tried to clarify. Regarding the fact that NAFLD is rarely isolated and that it is correlated with obesity, diabetes type 2, polycystic ovarian syndrome, obstructive sleep apnea, and some cognitive deficits, its pathophysiology and clinical development require more investigations. Suggestions for the treatment by the implantation of the intragastric balloon must be considered as one of the treating solutions in the future.

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Research Article

Predictive Value of Adiposity Level, Metabolic Syndrome, and Insulin Resistance for the Risk of Nonalcoholic Fatty Liver Disease Diagnosis in Obese Children

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Background. Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in obese children. Early diagnosis and treatment are essential for curing or slowing down the disease progression. The aim of the study was to assess the prevalence of NAFLD in this population and to identify anthropometrical and metabolic risk factors for NAFLD prediction and its development. **Material and Methods.** The study included 108 obese children. Anthropometric measurements, NAFLD diagnosis (based on ALT level and/or liver ultrasound), and metabolic syndrome (MS) components were assessed in all patients. Patients were divided into groups with and without NAFLD. **Results.** NAFLD was diagnosed in 49 (45%) patients with similar prevalence in boys (27; 55.10%) and girls [22 (44.9%), $p = 0.089$]. NAFLD patients had significantly greater waist circumference, WHR, and WHtR and significantly higher total cholesterol, triglyceride, and fasting insulin concentrations as well as higher glucose and insulin concentrations in 120 minutes of OGTT and higher HOMA-IR levels compared to group of patients without NAFLD. In NAFLD patients, MS was significantly more likely to be diagnosed than in group without NAFLD (40.82% versus 22.81%, $p = 0.04$), but among the MS components only hypertriglyceridemia was significantly more frequently diagnosed in the group with NAFLD ($p = 0.002$). Among analysed parameters the best independent risk factor for NAFLD was fasting insulin concentration with the cut-off point = 18,9 uIU/ml (AUC = 0.829). **Conclusions.** NAFLD is a very common disease in obese children. NAFLD predictive risk factors include increased waist circumference, elevated WHR and WHtR, and elevated total cholesterol, triglycerides, and fasting insulin as well as elevated glucose and insulin concentration in the OGTT and HOMA-IR index. NAFLD increases the risk of potential cardiovascular complications expressed by diagnosis of metabolic syndrome. The best independent predictive risk factor for diagnosing NAFLD in obese children is fasting insulin > 18.9 uIU/ml.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in children [1]. Due to the growing number of obese children the prevalence of fatty liver increases, and it was assessed in around one-third of clinical population [2]. NAFLD is defined as hepatic fat infiltration > 5% of hepatocytes, assessed by liver biopsy, after exclusion of excessive alcohol intake and other liver pathologies. In children NAFLD is, usually at the time of diagnosis, a simple steatosis, which is reversible, but also can initiate further stages of disease, such as nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. The pathogenesis of NAFLD

and its progression are complex process, called multiple-hit theory combining environmental (dietary habits, physical activity [3, 4]), molecular (lipotoxicity [5]), endoplasmic reticulum stress [6], mitochondrial dysfunction [7], organokines effect [8, 9], genetic (polymorphism involved in the onset and progression of the disease), and other factors like dysbiosis [7, 10, 11].

NAFLD not only in adults but also in children is associated with severe metabolic disorders as hypertension, dyslipidaemia, increased risk of type 2 diabetes, metabolic syndrome, and cardiovascular diseases [12, 13]. For many years, NAFLD has been perceived as a hepatic consequence of insulin resistance, but recent studies have shown that fatty

liver disease can precede with type 2 diabetes and metabolic syndrome and may even be a risk factor for their development [14, 15]. Due to biopsy limitations, hepatic steatosis is also diagnosed with biochemical tests like elevated alanine transaminase, or imaging methods, especially ultrasound. Despite the limitations, ultrasonography is characterized by high sensitivity (60–94%) and specificity (84–100%) for NAFLD detection [16]. Early diagnosis in the asymptomatic period and effective therapy implementation enable curing or, at least, slow down the progression of the disease.

The aim of the study was to assess the prevalence of NAFLD, as well as identifying additional predictive anthropometrical and metabolic risk factors for NAFLD development in the obese children.

2. Materials and Methods

2.1. Study Population. The study was prospective and included 108 children aged 6 to 18, hospitalized in our department between years 2012 and 2014, whose BMI exceeded 97 pc for sex and age, after informed consent. Children with acute infectious disease, chronic hepatitis of known etiology, using hepatotoxic drugs, or consuming alcohol were excluded from the study. A general medical examination, anthropometric measurements, laboratory tests, blood pressure measurements, and abdomen ultrasound imaging were performed in all subjects.

2.2. Anthropometric Evaluation and Body Composition. All anthropometric measurements were made in the morning, with fasting with empty bladder in the upright position. Subjects were barefoot and lightly dressed. Height and weight were measured to the nearest 0,1 cm and 0,1 kg, respectively, by using calibrated scale and Harpenden stadiometer. Waist and hip circumference were measured to the nearest 0,5 cm using standard technique with nonelastic tape, at the end of normal expiration. Waist circumference (WC) was measured at a point midway between the lower border of the ribs and the iliac crest, and hip circumference was measured at the widest part of the hip. BMI, waist-hip ratio (WHR), and waist-to-height ratio (WHtR) were calculated. Standard deviations scores for height and BMI were calculated using the LMS method based on Polish reference values [17].

Body composition was evaluated by bioelectrical impedance using the Tanita MC 980 MA device-multifrequency segmental analyser. Estimates of body composition were obtained from the equipped software. Patient stand barefoot on the marked electrodes and in straightened hands held the handles equipped with electrodes. The measurement of the tissue impedance through which the six-frequency (1 kHz, 5 kHz, 50 kHz, 250 kHz, 500 kHz, and 1000 kHz) and low intensity (<90 μ A) current was applied is painless and lasts for several seconds. Of the many variables, the following results were analysed: total body water in kg and % (TBW), fat mass in kg and fat%, fat-free mass in kg (FFM), muscle mass in kg, visceral fat in %, and basal metabolic rate in kJ (BMR). Moreover the following indicators were calculated: visceral fat to total body fat (visceral fat%/fat%), basal

metabolic rate per kilogram body weight (BMR/kg), and standard deviations scores for fat percentage (fat%Z-score = $2 * (\text{fat}\% - 50 \text{ pc fat}\%) / (98 \text{ pc fat}\% - 50 \text{ pc fat}\%)$) based on children reference values [18].

2.3. Liver Ultrasonography. Liver ultrasound examination was performed by two radiologists with Siemens Acuson Antares, convex transducer 5 MHz. Fatty liver was diagnosed on the basis of increased echogenicity of the liver parenchyma compared to the right kidney echogenicity.

2.4. Laboratory Assessment. Fasting venous blood sample was taken in the second day of hospitalization. Blood chemistry analyses, alanine transaminase (ALT), gamma glutamyltransferase (GGT), total cholesterol (Tch), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), and bilirubin, were performed in hospital laboratory by using standard methods. Oral glucose tolerance test (OGTT; 1.75 g/kg body weight, up to 75 g) was performed in all patients. Glucose and insulin concentration were measured during fasting and in 120 minutes of the test. In this study, elevated ALT levels were identified for ALT > 35 U/l in children aged 3–11 years and ALT > 40 U/l in children aged \geq 11 years. Hypertriglyceridemia was defined as fasting triglycerides \geq 150 mg/dl, and low HDL cholesterol as fasting HDL < 40 mg/dl in children aged < 16 years and <40 mg/dl for boys or <50 mg/dl for girls aged > 16 years [19]. Impaired fasting glucose (IFG) was defined as fasting glucose \geq 100 mg/dl.

2.5. Blood Pressure Measurements. Blood pressure (BP) was measured three times daily by the Korotkoff method using sphygmomanometer, on the right arm after 5–10 minutes of rest in the sitting position. The results were referred to Polish children reference values [20]. Patients with at least two measurements > 90 pc (high normal pressure) and patients with previously treated hypertension performed 24-hour ambulatory blood pressure monitoring using the Space-labs ABP. Hypertension was diagnosed when the systolic blood pressure was above 95 pc for more than 25% of the measurements.

2.6. Insulin Resistance and Metabolic Syndrome (MS). Insulin resistance was measured by homeostasis model assessment of insulin resistance (HOMA-IR) described by Matthews et al. [21], defined as follows: $\text{HOMA-IR} = \text{fasting glucose (mg/dl)} \times \text{fasting insulin (uIU/ml)} / 405$. The outcomes were referred to Caucasian obese children reference values [22].

We used the definition of the International Diabetes Federation (IDF) for the metabolic syndrome in children and adolescents [19]. The first criterion reached in all our patients was central obesity defined as WC > 90 pc for age and sex or above adult norms (>80 cm for women and >94 cm for men). If patients reached at least 2 of the other 4 criteria (TG concentration \geq 150 mg/dl or hypolipemic treatment; HDL levels < 40 mg/dl (for girls > 16 years, HDL < 50 mg/dl); elevated blood pressure (systolic > 130 mmHg or diastolic > 85 mm Hg) or hypertension; fasting glucose \geq 100 mg/dl; or type 2 diabetes) then the metabolic syndrome was diagnosed.

TABLE 1: Anthropometric characteristic of studied population.

Variable	All (N = 108)		Males (N = 50)		Females (N = 58)		ttest p value
	Mean	SD	Mean	SD	Mean	SD	
BMI z-score	2.40	0.48	2.36	0.47	2.43	0.50	0.463
height z-score	0.42	1.4	0.60	1.55	0.27	1.26	0.230
WC (cm)	103.9	12.9	108.17	12.06	100.16	12.49	0.001
Hip circumference (cm)	108	11.3	108.12	11.47	107.90	11.29	0.922
WHR	0.96	0.07	1.0	0.06	0.93	0.07	<0.001
WHtR	0.63	0.06	0.64	0.06	0.62	0.06	0.073
Fat%	39.27	6.79	37.17	7.36	41.08	5.71	0.002
Fat% z-score	3.06	1.03	2.84	0.87	3.31	1.14	0.017
Visceral fat/fat%	0.46	0.45	0.44	0.45	0.48	0.45	0.769
BMR (kJ)	7851	1233	8681	1056	7135	878	<0.001
BMR/kg (kJ/kg)	92	13.94	96.4	13.7	88.5	13.17	0.003

2.7. NAFLD Diagnosis. NAFLD was diagnosed based on ultrasound examination and/or elevated ALT.

2.8. Statistical Analysis. The statistical software (Statistica v. 12.5 and Microsoft Excel) was used in data analysis. Descriptive data were expressed as median, mean values, and standard deviations (SD) for continuous variables. Patients were divided into two groups: with and without NAFLD, which were then compared for anthropometric features, biochemical parameters, and metabolic syndrome components. Mann-Whitney U, ANOVA Kruskal-Wallis, Spearman rank correlation tests were used to compare continuous variables. Chi-square test was used for qualitative variables. The receiver operating characteristics (ROC) analysis was used to verify the characteristics of the independent variables and to assess an appropriate cut-off. Statistical significance was set at *p* value less than 0.05.

3. Results

The study included 108 obese children (50 boys) aged 6 years and 2 months to 17 years and 10 months. Mean age was 14.24 ± 2.73 SD. Mean age of girls was 14.75 ± 2.79 SD and of boys 13.9 ± 2.64 SD. Girls were about one year older than boys, but the difference was not statistically significant ($p = 0.074$). The age distribution of the examined group is shown in Figure 1. The only statistically significant difference was observed in the 16–18-year age group, where girls predominated. Table 1 gives the anthropometric and body composition characteristic of the study population stratified by gender. Males ($n = 50$) and females ($n = 58$) had similar BMI and height Z-scores while males had significantly higher mean WC and WHR. Among important anthropometrical data for obesity diagnosis, parameters of body composition males had significantly higher fat% and fat%Z-score; however no difference in visceral fat/fat% was observed.

3.1. NAFLD Prevalence. NAFLD was diagnosed in patients with hyperechogenic liver on ultrasound and/or elevated ALT concentration. Ultrasonographic features of fatty liver were

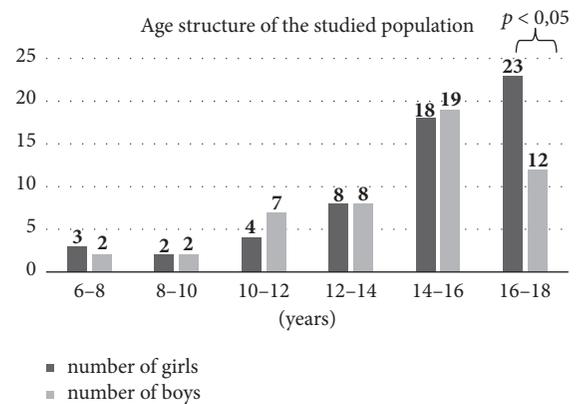


FIGURE 1: Sex distribution in particular age groups.

diagnosed in 34 patients (16 girls and 18 boys) and elevated ALT in 30 patients (8 girls and 22 boys). In 15 patients (2 girls and 13 boys) both were reported. Based on proposed criteria, two groups were identified: (I) NAFLD patients ($N = 49$; 45.4%) and (II) non-NAFLD patients with normal liver and normal ALT concentration ($N = 59$; 54.6%).

NAFLD was diagnosed more often in boys than in girls; however the difference was insignificant (55.1% versus 44.9%; $p = 0.089$).

3.2. Anthropometry and Body Composition. NAFLD patients did not differ significantly from patients without NAFLD in age, height, body weight, BMI, or BMI Z-score, while NAFLD was associated with a significantly greater waist circumference, WHR, and WHtR. Body composition analysis did not show statistically significant differences between groups in key obesity parameters such as fat mass, fat%, fat% Z-score, or visceral fat%. Groups differed significantly only in fat-free mass (FFM), total body water (TBW), and basal metabolic rate (BMR); however BMR per kilogram remained similar in both groups. The comparison of the two groups in terms of basic anthropometric and body composition parameters is presented in Table 2.

TABLE 2: Anthropometry and body composition. Comparison of patients with and without NAFLD.

Variable	NAFLD (N = 49)			Patients without NAFLD (N = 59)			p value (Mann–Whitney U test)
	Mean	SD	Median	Mean	SD	Median	
Age (years)	14.40	2.49	14.83	14.17	2.94	14.92	0.828
Waist (cm)	107.70	12.44	107.00	100.95	12.60	100.50	0.017
Hip (cm)	108.78	11.50	109	107.53	10.97	108	0.794
WHR	0.99	0.06	0.98	0.94	0.08	0.94	<0.001
WHtR	0.65	0.06	0.65	0.62	0.06	0.62	0.041
Height (cm)	166.10	11.95	166.40	162.70	12.41	161.50	0.127
Weight (kg)	92.22	22.78	89.00	84.53	19.74	84.80	0.126
BMI (kg/m ²)	32.97	5.29	32.00	31.49	4.82	31.60	0.243
BMI Z-score	2.45	0.48	2.38	2.37	0.48	2.36	0.479
Height Z-score	0.53	1.44	0.53	-0.573	0.566	-0.573	0.566
Fat%	39.40	6.34	38.90	39.15	7.23	39.60	0.897
Fat mass (kg)	36.61	11.58	36.50	33.44	10.91	34.60	0.214
FFM (kg)	55.62	14.01	54.60	51.08	12.68	48.90	0.047
FFM%	60.61	6.35	61.07	60.83	7.23	60.35	0.879
Muscle mass (kg)	52.40	13.72	51.80	48.57	12.13	46.60	0.094
TBW	40.72	10.26	40.00	37.38	9.28	35.80	0.047
TBW%	44.33	4.67	44.70	44.53	5.28	44.20	0.919
Trunk fat%	35.39	7.08	35.00	34.84	8.07	35.80	0.886
Fat% Z-score	3.15	0.99	3.04	2.98	1.07	2.92	0.577
Trunk/total	0.89	0.06	0.88	0.88	0.07	0.89	0.986
BMR	8158	1293	7880	7617	1144	7512	0.028
BMR/kg	90.89	12.33	86.17	92.80	14.85	88.57	0.597

TABLE 3: Biochemical analysis. Comparison of patients with and without NAFLD.

Variable	NAFLD patients (N = 49)			Patients without NAFLD (N = 59)			p value (Mann–Whitney U test)
	Mean	SD	Median	Mean	SD	Median	
Tch (mg/dl)	185.12	28.64	185.00	169.02	30.01	167	0.004
HDL (mg/dl)	45.34	10.26	43.90	47.77	11.38	45.85	0.298
LDL (mg/dl)	103.16	25.69	99.90	96.26	28.34	62.03	0.153
TG (mg/dl)	183.08	79.69	173.00	129.15	62.03	114.00	<0.001
glu 0 (mg/dl)	90.88	10.00	90.00	88.95	7.74	88.50	0.403
glu 120 (mg/dl)	122.04	22.68	121.00	109.91	19.84	109.00	0.007
ins 0 (μIU/ml)	28.48	21.54	23.15	12.86	6.76	12.00	<0.001
ins 120 (μIU/ml)	130.38	98.18	93.80	81.36	44.03	70.70	0.004
HOMA-IR	6.44	4.83	4.93	2.86	1.67	2.58	<0.001

3.3. *Biochemical Analysis.* Of the biochemical parameters, the serum concentration of total cholesterol, triglycerides, fasting insulin, glucose, and insulin in 120 minutes of OGTT and HOMA-IR were significantly higher in patients with NAFLD.

NAFLD was significantly more often diagnosed in patients with HOMA-IR exceeding reference values [22] than in children with the normal range of HOMA-IR (79% versus 28%, $p = 0.00$ for HOMA-IR > 90 percentile; 85% versus 15%, $p = 0.00$ for HOMA-IR > 97 percentile). A detailed summary of the biochemical parameters is presented in Table 3.

The ROC analysis was performed on variables that differentiated NAFLD patients from non-NAFLD ones. Based on the area under receiver operating curves (AUC) we

established a threshold of each variable that showed the highest accuracy for identifying children at high risk of NAFLD. Figure 2 shows a comparison of the ROC curves for the evaluated parameters. The largest area under the ROC curve was obtained for fasting insulin (AUC = 0.829, 95% CI 0.746–0.911) and for HOMA-IR (AUC = 0.817; 95% CI 0.733–0.901). The optimal cut-off point of the insulin level for diagnosing NAFLD was 18,9 uIU/l with the sensitivity of 75% and specificity of 87,3%. The performance of these variables in NAFLD prediction is summarized in Table 4.

3.4. *Metabolic Syndrome.* In the study population 34 (31.48%) patients were diagnosed with metabolic syndrome (MS). Of the metabolic syndrome components abdominal obesity and

TABLE 4: Diagnostic value of analysed variables in NAFLD prediction.

Variable	Symbol	AUC (95% CI)	Cut point	Sensitivity	Specificity	SE
Total cholesterol	Tch	0.660 (0.557–0.764)	197 mg/dl	44.9%	80.7%	0.053
Triglycerides	TG	0.713 (0.614–0.813)	161 mg/dl	61.2%	75.4%	0.051
Glucose in 120 min of OGTT	glu 120	0.654 (0.548–0.759)	112 mg/dl	70.2%	56.4%	0.054
Fasting insulin	ins 0	0.829 (0.746–0.911)	18.9 μ IU/ml	75%	87.3 %	0.042
Insulin in 120 min of OGTT	ins 120	0.666 (0.559–0.772)	129 μ IU/ml	38.3%	90.9%	0.054
Insulin resistance	HOMA-IR	0.817 (0.733–0.901)	4.089	70.8%	83.6%	0.043

TABLE 5: Prevalence of metabolic factors in NAFLD and non-NAFLD group.

MS components	Total (%)	NAFLD patients (N = 49)	Patients without NAFLD (N = 59)	p
WC > 90 pc	108 (100%)	49 (100%)	59 (100%)	NS
TG > 150 mg/dl or hypolipemic treatment	49 (45.37%)	30 (61.22%)	19 (32.20%)	0.002
HDL < 40 mg/dl (girls > 16 years; HDL < 50 mg/dl)	39 (36.11%)	20 (40.82%)	19 (32.20%)	NS
Hypertension	21 (19.44%)	11 (22.45%)	10 (16.95%)	NS
IFG or diabetes 2	13 (12.04%)	6 (12.24%)	7 (11.86%)	NS
MS	34 (31.48%)	20 (40.82%)	14 (23.73%)	0.04

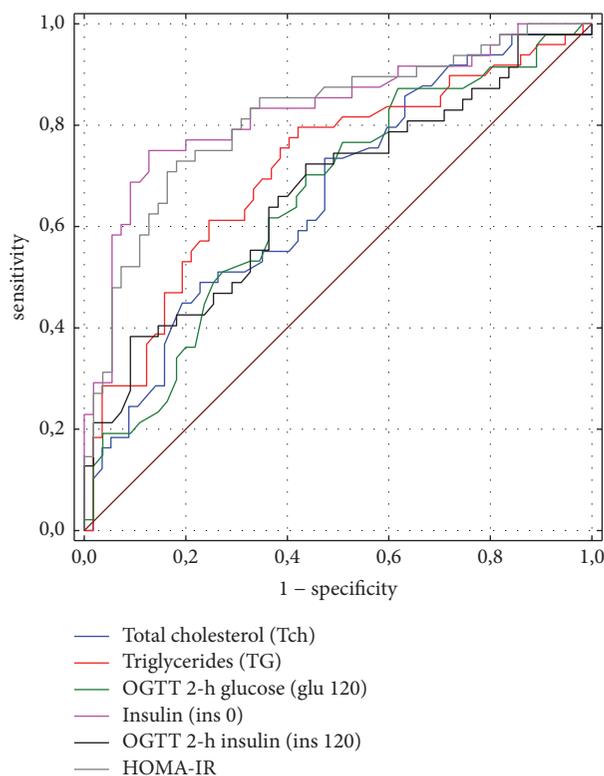


FIGURE 2: Comparison of ROC curves for parameters significantly differentiating patients with NAFLD from non-NAFLD patients.

hypertriglyceridemia were most often recognised. MS was diagnosed significantly more frequently in NAFLD group than in patients without fatty liver (40.82% versus 23.73%, $p = 0.04$), but of the MS components only hypertriglyceridemia was significantly more often diagnosed in NAFLD patients. The frequency of the MS and its components in the study population is presented in Table 5.

4. Discussion

We diagnosed NAFLD on the basis of elevated ALT and/or presence of steatosis on ultrasound. Both of these criteria are often used in noninvasive diagnosis of fatty liver disease. High sensitivity (60–94%) and specificity (84–100%) of ultrasonography make this examination suitable for screening [16]. Alanine aminotransferase (ALT) concentration due to wide availability and low cost is the basic marker used in liver diseases screening, including NAFLD. Interpretation difficulties are caused by different cut-off points proposed by each investigator like ALT > 52 U/L for girls and >72 U/L for boys in the Brazilian study [23], ALT > 50 U/L the in the Wiegand et al. study [24], or ALT > the laboratory standard in most authors' studies. On the other hand, we should be aware of low sensitivity of ALT at standard cut-off point (45 U/l); despite liver steatosis confirmed by imaging studies, in most pediatric patients, ALT levels remain normal. Therefore, a number of population studies suggest introducing new, sex-specific thresholds for ALT [25]: in children ALT < 25.8 IU/l for boys and ALT < 22.1 IU/l for girls in American study [26], ALT < 33 IU/l for boys and <25 IU/l for girls in Korean study [27], or ALT < 30 IU/l and <21 IU/l in Iranian study, respectively [28].

In this study we diagnosed NAFLD in 45% of patients. Such a high prevalence is caused by both group selection (obese children hospitalized for obesity) and diagnostic criteria. Fatty liver on ultrasound was reported in 34 (31.48%) patients, and elevated ALT levels were reported in 30 (27.78%) patients.

The authors of meta-analysis from 2015 [2], based on 56 clinical trials, assessed the prevalence of NAFLD in obese children on 34.2% with a prediction range from 5.2% to 83.1%. Researchers, however, emphasize significant differences in the methodology, diagnostic criteria, or accepted standards for the laboratory tests results. With the lowest frequency NAFLD was diagnosed in 2.3% patients by Brazilian

researchers [23], which is caused by the inclusion criteria: abdominal obesity defined as WC > 75 pc and significantly higher ALT (52 U/L for girls and ALT > 72 U/L for boys) as well as ethnic differences (only 29% of the children were Caucasian). As much as 83.1% of patients were diagnosed with NAFLD in the American study [29]; however, the group consisted of children who were qualified to bariatric surgery. The mean prevalence of NAFLD diagnosed on ultrasound in the previously cited meta-analysis was 41%, while that diagnosed on the basis of elevated alanine aminotransferase was only 13.7% [2].

Males have higher prevalence of NAFLD than females, which is confirmed by most studies [2, 24, 30], but not in the Italian study [31] where NAFLD was similar in both sexes (58% of boys and 46% of girls, $p = 0.31$). However, in that study prepubertal population was analysed, which was significantly younger than in other studies. In our study prevalence of NAFLD was similar in males and females, which could have come from the sex distribution in age groups (there were significantly more girls in the 16–18 age group, $p < 0.05$).

In our study NAFLD patients were similar to non-NAFLD ones in age and basic anthropometric parameters such as height, weight and even BMI, and BMI Z-score. There was also no difference in the most important parameters of the body composition (fat mass, fat%, fat% Z-score, or visceral fat%). Children with NAFLD had only significantly higher fat-free mass, total body water content, and BMR (but not BMR/kg); however, these parameters, due to their weight, age, or gender dependence, have little diagnostic value.

The only anthropometric parameters discriminating NAFLD patients from non-NAFLD, in our study, are waist circumference (WC) and the indicators associated with WC, WHR and WHtR. These are important, practical pieces of information indicating that BMI, even referred to reference values, is not sufficient for obese patients, while WC, which is still not a routine medical examination, may be an effective tool for detecting patients at risk of metabolic disorders, including NAFLD. Many studies have shown that waist circumference may be used in central obesity screening, cardiovascular risk assessment in children [32], and NAFLD diagnosis [33–35].

Similar results were obtained by Italian and Taiwanese researchers who did not observe differences in BMI and body composition between the groups with and without NAFLD, but waist circumference was significantly higher in NAFLD group [31, 34]. Denzer et al. also observed significantly greater waist circumference in patients with NAFLD (mean 111 cm versus 101 cm in patients without steatosis, $p < 0.0001$) but, in contrast to our results, NAFLD patients had significantly higher BMI-Z score (mean 2, 78 versus 2.56 in non-NAFLD, $p < 0.0001$) [30].

Considering the complexity of links between NAFLD, insulin resistance (IR), and type 2 diabetes, it is extremely difficult to find out whether NAFLD is the cause or the effect of insulin resistance. For many years, NAFLD has been treated as the hepatic consequence of IR, but recent studies suggest that hepatic steatosis may precede type 2 diabetes and metabolic syndrome and may even be a risk factor for their development [14, 15].

In our survey in diagnostics of carbohydrate metabolism fasting glucose remained useless, which was confirmed in other studies [31, 36]. Significant differences between groups were observed in fasting insulin concentration and glucose and insulin concentration in 120 minutes of OGTT. In our study, the highest diagnostic value for detecting NAFLD achieved fasting insulin with AUC = 0.829, and the cut-off point of 18.9 $\mu\text{IU/ml}$ with 75% sensitivity and 87.3% specificity diagnosed NAFLD. Studies in obese patients [31, 36, 37] confirm correlation of fasting insulin concentrations with NAFLD diagnosis; however there are no clear standards for this parameter, which makes it difficult to apply. In Shashaj et al. study [22] mean fasting insulin concentration in obese patients was 13.8 $\mu\text{IU/ml} \pm 9.4$, while in Pacifico et al. it was 10.6 $\mu\text{IU/ml}$ in obese children without steatosis and 15.6 $\mu\text{IU/ml}$ in obese children with NAFLD [36]. Much higher mean concentration of fasting insulin was observed by Korean researchers, 15.1 $\mu\text{IU/ml} \pm 6.0$ in obese children without NAFLD and 24.8 $\mu\text{IU/ml} \pm 14.6$ in children with NAFLD [37]. These results are comparable to our study (12, 86 $\mu\text{IU/ml} \pm 6.76$ in children without steatosis versus 28.48 $\mu\text{IU/ml} \pm 21.53$ in children with NAFLD).

NAFLD patients had significantly higher HOMA-IR values compared to non-NAFLD ones. Similar observations have been made by Denzer et al. [30] and D'Adamo et al., who confirmed that insulin resistance indexes were significantly associated with NAFLD independently of BMI [31]. In contrast Gökçe et al. concluded that neither mean HOMA-IR values nor the prevalence of insulin resistance was higher in NAFLD group [12].

It is proven that insulin resistance increases in obese patients; therefore separate standards are developed for the population of obese children [22]. Authors of this study emphasize that in obese children HOMA-IR > 75 pc is associated with an increased cardiometabolic risk defined as at least one of the following: hypercholesterolemia, hypertriglyceridemia, reduced HDL levels, and ALT > 40 U/l. They have also observed that HOMA-IR > 3.42 (AUC = 0.71) with 48.8% sensitivity and 81.3% specificity identifies cardiovascular risk in obese patients. Referring our results to the proposed standards [22] we proved that NAFLD is significantly more common in patients with increased HOMA-IR compared to obese patients with normal HOMA-IR (79% versus 28%, $p = 0.00$ for HOMA-IR > 90 pc; 85% versus 15%, $p = 0.00$ for HOMA-IR > 97 pc), and HOMA-IR > 4.089 is a good indicator of NAFLD (AUROC = 0.817, sensitivity = 70.8%, specificity = 83.6%, and 95% CI = 0.733).

Metabolic syndrome (MS) in adult patients increases the risk of cardiovascular disease. In the pediatric population such conclusions are not clear. Due to both lack of long-term follow-up of pediatric patients and different criteria for MS, some authors suggest that the prevalence of individual components of metabolic syndrome in the pediatric population would be more relevant for assessment of the cardiovascular risk than the diagnosis of metabolic syndrome [38, 39]. In the studied population, based on IDF criteria [19], 34 (31.48%) patients were diagnosed with MS, which is comparable to other studies using the same criteria (Strojny et al., 29% [40]). In contrast, Manco et al. diagnosed MS only in 10%

of children with NAFLD, which may be because only 65% of the children in the study group were obese [35]. In our study, patients with MS were more likely to have NAFLD than patients without MS (60.6% versus 39.19%, $p < 0.05$), but no correlation was found between the number of metabolic syndrome criteria and the prevalence NAFLD ($p = 0.052$), which may be caused by the fact that only fasting glucose but not the insulin resistance is considered in the IDF criteria for MS.

5. Conclusions

NAFLD is a very common disease in obese children. NAFLD risk factors include increased waist circumference, elevated WHR and WHtR, and elevated total cholesterol, triglycerides, and fasting insulin as well as glucose and insulin concentration in 120 min of OGTT and HOMA-IR index. NAFLD increases the risk of potential cardiovascular complications expressed by diagnosis of metabolic syndrome. The best independent risk factor for diagnosing NAFLD in obese children is fasting insulin concentration > 18.9 uIU/ml.

In our study, we focused on the diagnosis of obese children in the context of metabolic disorders, especially related to obesity and fatty liver disease. The prevalence of NAFLD and other metabolic disorders in the study population indicates the need to improve diagnostics in obese children already at primary health care level. Simple diagnostic methods such as waist circumference measurement and fasting plasma insulin concentration may contribute to the early identification and prediction of patients at risk of NAFLD and other metabolic complications. Appropriate therapy and lifestyle change in these patients and their families will help to prevent the negative effects of obesity in the future.

Data Availability

The data that support the findings of this study are available from the corresponding author [Pawel Matusik], upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Clinical Study

The Effects of Extra Virgin Olive Oil on Alanine Aminotransferase, Aspartate Aminotransferase, and Ultrasonographic Indices of Hepatic Steatosis in Nonalcoholic Fatty Liver Disease Patients Undergoing Low Calorie Diet

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Background. Coronary artery disease is the most common cause of death in the patients with nonalcoholic fatty liver disease (NAFLD). Studies have shown that there is a strong relation between the increase in the aminotransferase levels and fat accumulation in the liver with cardiovascular complications, independent of all aspects of the metabolic syndrome. This study aimed to examine the effect of virgin olive oil on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the severity of steatosis in the NAFLD patients undergoing a weight-loss diet. **Methods.** This clinical trial was carried out on 50 patients with nonalcoholic fatty liver (mean age of 45.91 ± 9.61 years, mean BMI of 29.7 ± 0.58 Kg/m²) and the subjects were randomly assigned to the olive oil group (receiving the equivalent of 20% of their total daily energy requirement from olive oil) or the control group (with normal consumption of oil) for 12 weeks. All the patients received a hypocaloric diet during the study. At the beginning and the end of the study, the serum levels of ALT and AST and liver steatosis were measured. **Findings.** A significant decrease in the level of ALT enzymes was observed in the control group at the end of the study ($P = 0.004$). In the olive oil group, both enzymes decreased compared to baseline measurements ($P < 0.01$). There were significant differences in the ALT and AST levels between the two groups ($P < 0.02$). The severity of liver steatosis did not change significantly during the study. **Conclusion.** The consumption of a low calorie diet enriched with olive oil, along with slight weight reduction, reinforces the desired effects of weight loss in improving the levels of the hepatic enzymes.

1. Introduction

At present, nonalcoholic fatty liver disease (NAFLD) is the most common cause of elevated serum aminotransferase [1]. In fact, elevated serum alanine aminotransferase (ALT) not only is a consequence of the NAFLD but also predicts the progression of the disease [2]. Recent findings point out that NAFLD may be linked to an increased risk of cardiovascular disease (CVD), which is the most common cause of overall

mortality [3, 4]. Many long-term follow-up studies of NAFLD found a strong link between mortality related to the coronary artery disease and NAFLD. Another common reason for mortality in the NAFLD patients is hepatic failure, especially in those with nonalcoholic steatohepatitis (NASH) [5–7]. The increased levels of the liver enzymes including ALT, aspartate aminotransferase (AST), and γ -glutamyl transferase (GGT) are the markers of NAFLD and the occurrence of CVD events in both nondiabetic subjects and the patients with type

2 diabetes [8]. Studies have also shown that ALT predicts cardiovascular events, early carotid atherosclerosis [9, 10]. This suggests that NAFLD is associated with coronary heart disease (CHD), independent of the other features of the metabolic syndrome. The studies showed that the prevalence of NAFLD in Iran was relatively high and the people with NAFLD had a higher risk of 10-year CVD events than the individuals without NAFLD [4, 11].

Animal models and human studies suggest that the dietary factors play a key role in the progression of NAFLD. In particular, the amount and type of dietary fat can affect fatty infiltration and lipid peroxidation in NAFLD [12, 13]. There is little research on the effects of the type of dietary fat in NAFLD [14]. Recently, the Mediterranean diet has received attention as a diet that prevents NAFLD and cardiovascular disease. It is known that olive oil, which is rich in the monounsaturated fatty acids (MUFAs), is responsible for the major part of the beneficial effects of the Mediterranean diet [15–17].

Animal studies indicated that olive oil consumption leads to an increase in the release of the triglycerides from the liver and a decrease in the flux of free fatty acids (FFAs) from the peripheral adipose tissue back to the liver [18]. In rats, a diet rich in olive oil led to the remission of hepatic steatosis [19]. In other animal studies, liver damage was found to be decreased in rats receiving olive oil, compared to those given polyunsaturated oil [20]. However, the role of MUFA or olive oil in human NAFLD is yet to be demonstrated.

Some human studies have been conducted on the effects of a high-fat diet (40%) on the serum ALT enzyme and the severity of steatosis in patients with type 2 diabetes [21, 22].

There is only one study on the effects of olive oil in patients with NAFLD who were given a low-fat diet (20% fats) [23]. In this study no significant changes were found in the ALT and AST levels.

Considering the high prevalence of NAFLD in Iran, we have tried to examine the effects of extra virgin olive oil in a normal fat diet (30%) on the serum levels of the ALT and AST enzymes and on the severity of steatosis in the NAFLD patients on a weight-loss diet.

2. Materials and Methods

2.1. The Subjects. This clinical trial was carried out on 50 patients (19 women and 31 men) with nonalcoholic fatty liver in Tehran, Iran. The mean age was 45.91 ± 9.61 years, and the mean BMI was 29.7 ± 0.58 Kg/m². The clinical inclusion criteria were the increase of the AST and ALT enzymes (U/L < 30 in men and <20 in women), the elimination of all other causes for the increase in the liver enzymes (other liver diseases), age of 20–65 years, BMI of 25–40 kg/m², no use of hepatotoxic medicines, no history of ≥ 30 gr/d alcohol consumption, no CVD, no diabetes, no pregnancy or breast feeding, no smoking, no consumption of mineral and multivitamins supplements, no consumption of olive products, and lipid-lowering medicines in the last three months. All the subjects gave their informed consent in writing. The trial was approved by the TUMS Research Ethical Committee

and was registered in the Iranian website for clinical trials (<http://www.irct.ir>, code: IRCT201111022709N20).

2.2. Sample Size. The sample size was calculated based on the only published paper in this area at the time of this study, which showed that a modified Mediterranean diet (rich in olive oil) caused a reduction in the ALT levels in obese patients with type 2 diabetes [21]. To determine the outcome with type one (α) and type two errors (β) of 0.05 and 0.20 (power = 80%) and 10% dropouts, 25 subjects in each group were recruited.

2.3. The Study Design. The study was a randomized, single-blind trial. The patients were randomly chosen by the qualified experts. The sample was recruited from the visiting patients in the gastroenterology ward of Imam Khomeini's Training and Treatment Hospital in Tehran, Iran. Then, the necessary briefing and the aims of the study were given to the subjects, and they were requested not to use olive oil for 10 days before starting the study. Then, the patients were divided into two groups by using the method of random allocation. The olive oil group received the hypocaloric diet enriched with olive oil (20% of total energy intake) while the control group received the hypocaloric diet with normal fat. None of the participants know about the other group and alternative treatment.

2.3.1. Experimental Design for the Weight-Loss Diet. At the beginning of the study, the weight-loss diet was set with an objective of 5% weight reduction during three months of the study. The daily energy intake recommendations were 50% carbohydrates, 20% protein, and 30% fats for both groups. First, the energy required by each individual was calculated on the basis of their age, weight, and height and the gram quantity of each macronutrient was estimated based on the information. Then, the personalized diet was set, and different food groups and the food-exchanging table were explained to the subjects.

In this diet, 20% of the total fat (30%) was allocated to olive oil or the usual culinary fat, for the olive oil group and the control group, respectively. The remaining 10% of the required daily amount of fat was provided from the other nutrition groups such as dairy, meats, and nuts. Necessary training was given to the patients in the olive oil group for the correct way of consumption of the olive oil at the beginning of the study. The patients in both groups were asked not to change the advised diet and their level of physical activities.

2.3.2. The Preparation Method for Olive Oil. The virgin olive oil used in this study belonged to the Eteka brand (Roudbar, Iran), affiliated to the Khoramshahr Extraction oil company. The required oil dosage was allocated and supplied to each patient every week. In order to reduce the bias in the consumption amount of oil, identical measuring mugs were given to each patient with the required oil amount printed on it.

TABLE 1: Demographic characteristics of the study subjects.

Variables	Groups		P value
	Olive oil (n = 25)	Control (n = 28) ^{††}	
Sex			
Male	13 (61.9%)	13 (61.9%)	0.993**
Female	8 (38.1%)	9 (40.9%)	
Age (Year)	46.14 ± 8.44	45.68 ± 10.8	0.87*
Disease duration (Year)	7.16 ± 2.4	6.91 ± 2.7	0.72*
Type of oil			0.83**
Nonhydrogenated oil [†]	15 (71.4%)	16 (73.76%)	
Hydrogenated oil	2 (9.5%)	3 (13.6%)	
Both	4 (9.1%)	3 (13/6%)	

* P value reported based on Independent Sample *t*-test; ** P value reported based on Chi-Square test. Quantitative data represented as mean ± SD or median (min-max). Qualitative data reported as frequency (percentage). [†]In all of the patients using nonhydrogenated oil was sunflower oil. ^{††}Was given usual daily consuming oil.

2.4. Measurements

2.4.1. Demographic Data. The demographic questionnaires were completed by interview. The height was measured by using the stadiometer attached to the scale with an accuracy of 0.5 cm without shoes; the weight was also measured by the Seca scale, with an accuracy of 0.5 kg, in the fasting state, and with minimum clothing without shoes. The waist circumference was measured by using the strip meter, with an accuracy of 0.5 cm, with minimum clothing in the standing position at the beginning and the end of the study. The body mass index (BMI) was calculated using weight in kg divided by meters squared. All the patients' medical and drug history were recorded. The level of physical activities was evaluated by using the physical activity international questionnaire.

2.4.2. Dietary Intake. The food record questionnaires were completed over three days (two normal days and one holiday) at the beginning and at the end of the study to estimate the consumption of energy, carbohydrates, protein, fat, vitamins C and E, beta carotene, zinc, selenium, and fiber. The nutrition information was analyzed with the Nutritionist IV software.

2.4.3. Biochemical Assessment. From each patient, 10 cc venous blood sample was taken from the vein in the left arm, after 12–14-hour fasting. The blood samples were taken with the patients in the sitting position, in the Laboratory of Imam Khomeini's Training and Treatment Center, Tehran, Iran. The blood serum was immediately separated using the centrifuge in the 3000th (temperature of 4°C) cycle. The AST and ALT hepatic enzymes in the samples were measured immediately with the enzymatic colorimetric method.

2.4.4. Ultrasound Imaging of the Liver. The severity of the steatosis was measured with an ultrasound in the afternoon, eight hours after a light breakfast. The liver ultrasound was carried out with a 3.5-MHz curvilinear probe by a radiologist. The patients were classified into three groups based on the fat accumulation in their livers, that is, slight, moderate, and severe degrees.

2.5. Statistical Analysis. The Statistical Package for the Social Sciences software (version 20, SPSS Inc., Chicago, IL, USA) was used to analyze the data. The Kolmogorov-Smirnov test was carried out to test the normality of the distribution. All variables were reported as mean ± standard deviation (SD). The Chi-Square's test and the independent *t*-test was used for analysing the variables such as physical activity, the type of medicines consumed, the type of edible oil used, and the severity of steatosis between two groups. Within each group, the comparisons were done by the paired-sample *t*-test and by the McNemar's test variables. A *P* < 0.05 was considered statistically significant.

3. Results

3.1. Demographic Data. Out of the 50 NAFLD patients who participated in this study, 4 patients were eliminated from the olive oil group and 3 patients were removed from the control group. A total of 43 patients completed the trial. The mean age of the subjects was 46.14 ± 8.44 and 45.68 ± 10.8 in the olive oil group and in the control group, respectively. Moreover, the mean BMI in the olive oil group was 29.64 ± 3.93 and in the control group was 29.9 ± 3.77 kg/m². There were no significant differences in gender, age, duration of disease (Table 1), weight, BMI, and waist circumference (Table 2) between the two groups at the beginning and the end of the study. Given the energy limitation imposed at the beginning of the study, a significant weight reduction of 3.45 kg (4.33%) was observed in the olive oil group and a weight reduction of 2.89 kg (3.54%) was seen in the control group at the end of the study (*P* < 0/001) (Table 2).

As shown in Table 3, there was a significant decreased intake in total energy, carbohydrates, proteins, fat, PUFA, and saturated fatty acids (SFA) in each group and a significant difference in the poly unsaturated fatty acids (PUFA) and the monounsaturated fatty acids (MUFA) intake between the two groups (*P* < 0/001).

3.2. AST and ALT Levels and the Severity of Steatosis. There was no significant difference at the beginning of the study in the serum AST and ALT levels between the two groups. At the

TABLE 2: Anthropometric measurements at baseline and the end of study.

Variables	Groups		P value
	Olive oil (<i>n</i> = 25)	control (<i>n</i> = 28)	
W at baseline (cm)	79.65 ± 11	81.65 ± 13.6	0.58*
W at end-of-trial (cm)	76.2 ± 10.1	78.7 ± 12.9	0.47*
P value	<0.001**	<0.001**	
BMI at baseline (kg/m ²)	29.64 ± 3.93	29.9 ± 3.77	
BMI at end-of-trial (kg/m ²)	28.4 ± 3.91	29.13 ± 3.8	0.81*
P value	<0.001**	<0.001**	0.68*
WC at baseline (cm)	103.8 ± 10.81	104.18 ± 10.62	0.92*
WC at end-of-trial (cm)	100.61 ± 10.1	102.13 ± 10.2	0.63*
P value	<0.001**	<0.001**	

* *P* value reported based on Independent Sample *t*-test; ** *P* value reported based on Paired *t*-test. Quantitative data represented as mean ± SD or median (min-max). W = weight, BMI = body mass index, and WC = waist circumference.

end of the study, a significant decrease was seen in the ALT and AST levels in the olive oil group ($P < 0.01$), compared to the control group. Moreover, there was a significant difference in both enzymes between the two groups at the end of the study ($P < 0.05$). Although the intragroup liver fat assessment revealed an improvement in both groups (more in the olive oil), there was no significant statistical difference in steatosis between the two groups at the end of the study (Table 4).

4. Discussion

In the present study, there was a significant decrease in weight and the ALT and AST levels observed at the end of the study, in both the olive oil group and the control group. Moreover, decrease in the ALT and AST levels in the olive oil group was significantly higher than the control group.

Weight reduction is the first treatment line in the patients suffering from nonalcoholic fatty liver and can motivate the improvement of steatosis and the aminotransferase levels [24]. One study has observed that 5% of weight decrease is enough to decrease the serum's ALT value and to improve steatosis, while a minimum of 9% weight loss is necessary for a significant improvement of NASH [25]. Another study indicated that the patients with more than 7% of their base weight reduction experienced significant improvement in steatosis, inflammation, and the score of NASH tissue activity compared to the patients whose weight loss is less than 7% [26]. Moreover, a weight-loss diet with a goal of 8% initial weight decrease in obese women for a period of 3 to 6 months showed that the effect of weight loss on the improvement of steatosis depends on the rate of weight loss and the initial content of liver fat [27]. In our research, none of the patients had a high or severe steatosis at the beginning of the study and the weight loss was less than 7% in both groups at the end of the study.

Dietary components, particularly the type and the amount of fats, are crucial for liver fat accumulation and are responsible for 15% of the liver fat content. The dietary fats can exert their role in liver steatosis both directly and indirectly (via its influence on adipose tissues) [28]. The studies carried out in this field are limited to the survey of the effects of the modified Mediterranean diet with high fat content and

MUFA and its comparison to a low-fat diet in the patients with insulin resistance.

Our research is the first study that has surveyed the effects of the MUFA (from olive oil source) in a diet with normal fat content (30%) administered to the patients with nonalcoholic fatty liver. In one study a balanced-fat diet rich in olive oil rather than sunflower oil after one month leading to a decrease in steatosis and the hepatic enzymes in rats was observed. In human, one study reported that a high-fat (45%) and high-MUFA diet lead to the decrease of the ALT enzymes [21]. These findings are in concurrence with our study. In another study, the patients with NAFLD in the intervention group received a high-MUFA diet (olive oil or canola) for six months [23]. The total content of fat in their diet was 20% of the daily energy intake. Contrary to our study, the daily consumption of 20 g of olive oil had no effect on the serum aminotransferase. The low-fat diet in this study could be a reason for the significant decrease in steatosis. However, the improvement in liver fat content was not adequate for a significant decrease in the aminotransferases.

In a study in the patients with type 2 diabetes it was observed that the percentage of liver fat content in the MUFA-receiving groups with or without exercise had a significant decrease as compared to the groups receiving carbohydrate (CHO) with or without exercise [22].

The beneficial effects of the MUFAs on the hepatic fat content can be explained by the more rapid oxidization of the MUFAs than the saturated fatty acids in the postprandial phase. The more favorable MUFAs deposit in the adipose tissue rather than in the liver following a diet rich in MUFAs may help avoid fat deposition in the liver [29]. In addition, a high-MUFA diet stimulates the activity of lipoprotein lipase more than a diet rich in saturated fats that leads to increase in clearance of circulating triglyceride-rich lipoproteins [30]. In addition to type, the amount of fat also plays a role in the pathogenesis and, probably, in the treatment of fatty liver as well. In our study, the amount of MUFA was less than 20%.

On the other hand, recent studies on nutritional genomics supported a key role of gene-diet interaction in NAFLD development [31]. For example, it is suggested that the obesity-associated (FTO) gene levels in the liver are involved

TABLE 3: Dietary intake of study participants, at baseline and after intervention.

Variables	Groups	Before Mean \pm SD	After Mean \pm SD	<i>P</i> value [■]
Energy (Kcal/day)	Olive oil	2613.8 \pm 662.3	1756.5 \pm 538	<0.001
	Control	2449.2 \pm 723.2	1695 \pm 527.1	0.001
	<i>P</i> value*	0.44	0.7	
Protein (g/day)	Olive oil	79.5 \pm 24.28	63.92 \pm 23.8	0.001
	Control	82.68 \pm 23.18	61.5 \pm 22.13	0.005
	<i>P</i> value*	0.69	0.74	
Carbohydrates (g/day)	Olive oil	374.16 \pm 79.1	252.5 \pm 53.7	0.004
	Control	334.1 \pm 62.6	246.6 \pm 49.3	0.005
	<i>P</i> value*	0.22	0.832	
FAT (g/day)	Olive oil	92.41 \pm 55.38	57.3 \pm 23.18	0.001
	Control	86.03 \pm 36.64	55.33 \pm 20.1	<0.001
	<i>P</i> value*	0.58	0.59	
SFA (g/day)	Olive oil	23.94 \pm 9.5	13.67 \pm 8.18	0.003
	Control	28.93 \pm 8.45	14.03 \pm 6.09	0.001
	<i>P</i> value*	0.411	0.870	
MUFA (g/day)	Olive oil	24.95 \pm 9.28	29.27 \pm 10.76	0.47
	Control	23.53 \pm 10.81	[†] 13.45 \pm 6.56	0.19
	<i>P</i> value*	0.873	<0.001	
PUFA (g/day)	Olive oil	37.88 \pm 14.63	12.43 \pm 4.36	0.002
	Control	32.52 \pm 11.8	[†] 26.3 \pm 8.4	0.003
	<i>P</i> value*	0.275	<0.001	
Fiber (g/day)	Olive oil	12.49 \pm 5.8	10.94 \pm 6.5	0.333
	Control	13.4 \pm 5.32	11.69 \pm 6.23	0.291
	<i>P</i> value*	0.595	0.070	
Beta-carotene (μ g/d)	Olive oil	245/7 \pm 57.28	226.68 \pm 30.76	0.234
	Control	239.23 \pm 95.81	218.82 \pm 72.54	0.171
	<i>P</i> value	0.708	0.895	
Vitamin E (mg/day)	Olive oil	5.1 \pm 3.5	2.53 \pm 2.64	0.174
	Control	4.31 \pm 3.1	2.16 \pm 1.79	0.122
	<i>P</i> value*	0.634	0.587	
Vitamin C (mg/day)	Olive oil	61.25 \pm 42.95	70.95 \pm 32.95	0.621
	Control	63.9 \pm 22.72	68.05 \pm 32.51	0.404
	<i>P</i> value*	0.863	0.761	
Selenium (mg/day)	Olive oil	0.09 \pm 0.04	0.07 \pm 0.04	0.134
	Control	0.13 \pm 0.17	0.12 \pm 0.12	0.823
	<i>P</i> value*	0.366	0.277	
Zinc (mg/day)	Olive oil	7.04 \pm 4.62	10.94 \pm 6.5	0.895
	Control	8/04 \pm 4.9	11.69 \pm 6.23	0.767
	<i>P</i> value*	0.711	0.615	

* *P* value reported based on Independent Sample *t*-test; [■] *P* value reported based on Paired *t*-test; SFAs = saturated fatty acids, PUFAs = polyunsaturated fatty acids, and MUFAs = monounsaturated fatty acids.

in oxidative stress and lipid deposition, which characterize NAFLD [32]. The level of FTO gene expression is related to the level of dietary macronutrients [33]. Interestingly, the FTO genotype can affect the success of lifestyle interventions in the prevention and treatment of obesity [34].

Moreover, the observed difference may be related to the method of measuring the fatty contents [35]. The NMR (Nuclear Magnetic Resonance) or the spectroscopic golden

standard method is used for measuring the existing fat percentage in the liver. It has high precision and accuracy and is considered as the strength of the mentioned study, as described by the researcher. However, in our study, ultrasonography was used because of its cost effectiveness and prevalence. This method has some limitations, including the fact that the results obtained by it depend on the mastery and expertise skills of the operator and the detecting sensitivity

TABLE 4: Aminotransferase and severity of steatosis at the start and the end of study.

Variables	Groups	Before Mean ± SD	After Mean ± SD	<i>P</i> value [■]
ALT (IU/dl)	Olive oil	48 ± 12.9	35.71 ± 11.33	<0.001
	Control	50.82 ± 10.37	46.18 ± 10.26	0.004
	<i>P</i> value*	0.43	0.003	
AST (IU/dl)	Olive oil	34.53 ± 5.3	26.1 ± 5.4	<0.001
	Control	34.68 ± 8.9	32.28 ± 2.2	0.25
	<i>P</i> value*	0.94	0.002	
<i>P</i> value [Ⓞ]				
Steatosis <i>N</i> (%)				
Slight	Olive oil	10 (47.61%)	15 (71.42%)	0.008
Moderate		11 (52.38%)	6 (28.57%)	
Severe		0	0	
Slight	Control	7 (31.81%)	10 (45.46%)	0.17
Moderate		15 (68.18%)	12 (54.54%)	
Severe		0	0	
	<i>P</i> value**	0.23	0.13	

* *P* value reported based on Paired Sample *t*-test. [■]*P* value reported based on Independent Sample *t*-test. [Ⓞ]*P* value reported based on McNemar. ** *P* value reported based on Chi-Square. *P* < 0.05: significant.

of ultrasonography decreases with a degree of fat infiltration less than 30% [2]. Considering the low grade of steatosis in our patients, in contrast to the Nigma study where the patients had higher liver fat, the use of ultrasonography may prevent the obtaining of accurate information on the changes of liver steatosis. Another limitation of this study was sample size and short follow-up duration. A larger group of patients and longer follow-up period are needed to confirm the results.

5. Conclusion

In conclusion, the results of this study suggest that normal fat percentage (30%) in a diet containing olive oil (consumption the equivalent of 20% of total calorie intake from virgin olive oil) along with slight weight loss (approximately 5%) reinforces the desired effects of weight loss in improving the levels of the ALT and AST enzymes.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Clinical Study

The Relationship between NAFLD and Sarcopenia in Elderly Patients

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Aim. Previous studies have shown that individuals with low muscle mass exhibit an increased risk of nonalcoholic fatty liver disease (NAFLD). In this study, we investigated the association between NAFLD and sarcopenia in elderly patients. **Methods.** We classified the participants into sarcopenia/nonsarcopenia groups based on dual-energy X-ray absorptiometry (DXA), muscle strength (grip strength), or/and physical performance (6 m usual gait speed). We diagnosed NAFLD by ultrasonography combined with the history of alcohol intake. Logistic regression analysis was used to assess the correlation between sarcopenia and NAFLD. **Results.** NAFLD was significantly less frequent in the sarcopenia group than in the nonsarcopenia group ($P < 0.01$). However, NAFLD was neither an independent risk factor nor a protective factor for sarcopenia. **Conclusions.** NAFLD is not independently associated with sarcopenia.

1. Introduction

Many challenges have emerged from the aging of society, and several growing health problems related to aging, including sarcopenia, need to be addressed by geriatric researchers. The term sarcopenia is derived from the Greek word for the loss of flesh and was first suggested by Rosenberg in 1989 [1]. Owing to an increasing number of basic and clinical studies, the definition of sarcopenia has been refined. In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) reported sarcopenia as “a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death” [2].

Nonalcoholic fatty liver disease (NAFLD) is a genetic environment-associated metabolic stress-related disease, and it has replaced viral liver diseases as the most common liver disease around the world. For example, the Third National Health and Nutrition Examination Survey in the United States showed that the prevalence rate of NAFLD was 19.0% [3]. Recent studies show that NAFLD and sarcopenia share

common pathological and physiological mechanisms; furthermore, skeletal muscle mass index (SMI) and hepatic steatosis are negatively correlated [4, 5].

In this study, we focused on NAFLD in elderly patients. According to the latest criterion, sarcopenia is diagnosed through a combination of muscle mass index, grip strength, and 6 m usual gait speed. The correlation between sarcopenia and NAFLD was discussed through a cross-sectional study. Similar studies had not been performed until now.

2. Materials and Methods

Materials. The subjects signed informed consent approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University. From June 2014 to June 2017, in the First Affiliated Hospital of Chongqing Medical University, inpatients older than 60 years at the geriatric department and endocrinology department, who had a stable condition, were included in the study. Patients with New York Heart Association (NYHA) class III disease, acute exacerbation of chronic obstructive pulmonary disease (AECOPD), and

acute stroke, among others, were excluded. Patients who had renal replacement therapy, stage 5 chronic kidney disease (estimated glomerular filtration rate < 15 mL/min), history of organ transplant, extrahepatic fibrosis, or secondary causes of fatty liver or patients who had taken immunomodulators within the preceding 6 months were also excluded. Dual-energy X-ray absorptiometry (DXA) and abdominal ultrasound were performed under the observation of routine physicians. Tests for 6 m usual gait speed were also performed. According to the recommendations of American Society of Hand Therapists (ASHT) [6], the left and right hands were each measured with Jamar digital hand dynamometer three times, and the maximum value was used.

2.1. Clinical and Laboratory Measurements. The patients who had completed DXA, tests for upper grip strength, and 6 m walking speed were selected. According to the recommendations of the Asian Working Group for Sarcopenia by using cutoff values for appendicular skeletal muscle mass/height² (7.0 kg/m² for men and 5.4 kg/m² for women by DXA), handgrip strength (<26 kg for men and <18 kg for women), and usual gait speed (<0.8 m/s), we divided the patients into a sarcopenia group and a nonsarcopenia group. According to the Fatty Liver and Alcoholic Liver Disease Study Group of Chinese Liver Disease Association along with the World Gastroenterology Organization Global Guidelines [7], the clinical and imaging diagnosis of NAFLD included the exclusion of significant alcohol consumption (≥20 g/d) and demonstration of hepatic steatosis by liver ultrasound in the presence of metabolic risk factors and other causes of hepatic steatosis or other chronic liver diseases.

Other Materials. After the patients had fasted for 8~12 h, their height, weight, and seated blood pressure were measured, and peripheral venous blood was obtained and analyzed using an automatic biochemistry analyzer to measure the levels of blood uric acid, alanine, aminotransferase (ALT), aspartate aminotransferase (AST), hypersensitive C-reactive protein (hs-CRP) and glycosylated hemoglobin (HbA1c), creatinine, total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), and high-density lipoprotein (HDL). Physicians collected medical history. Additionally, body mass index (BMI) was calculated according to the following formula: BMI = weight (kg)/height (m)².

2.2. Statistical Analysis. The software Statistical Analysis System 8.0 was used for all data analysis. Continuous variables are represented by the mean ± standard deviation. Chi-square test was used for categorical variables, and Student's *t*-test was used for continuous variables to assess statistical significance of differences between two groups. Logistic regression analysis was used to assess the correlation between sarcopenia and NAFLD, HbA1c, hs-CRP, BMI, age, and sex. *P* < 0.05 was considered statistically significant.

3. Results

A total of 494 patients aged 60 to 96 years were enrolled in this study (216 males and 278 females). The average age of the

TABLE 1: Clinical characteristics of the study population.

	Sarcopenia	Nonsarcopenia
Number	158	336
Male/female	87/71	129/207
Hyperuricemia (%)	18.99%	20.24%
Diabetes (%)	74.05%	80.4%
Hypertension (%)	56.32%	56.55%
ALT (U/L)	22.85 ± 21.42	22.08 ± 16.77
AST (U/L)	23.14 ± 14.71	22.16 ± 15.44
HbA1c	7.79 ± 2.33	8.2 ± 2.36
TC (mmol/L)	4.38 ± 2.80	4.32 ± 1.53
TG (mmol/L)	1.50 ± 1.83	1.71 ± 1.65
HDL (mmol/L)	1.26 ± 0.39	1.21 ± 0.38
LDL (mmol/L)	2.47 ± 0.90	2.58 ± 0.97
NAFLD (%)	22.15%*	37.20%
Excess hs-CRP (%)	48.10%*	30.95%
Age (years)	73.75 ± 8.52*	70.12 ± 6.95
BMI (kg/m ²)	22.13 ± 2.97*	25.02 ± 3.34

* *P* < 0.05.

TABLE 2: Correlation coefficients between sarcopenia and NAFLD.

	NAFLD	
	<i>R</i>	<i>P</i>
Sarcopenia	-0.15	0.001

patients was 71.28 years. There were 158 cases (87 males and 71 females) in the sarcopenia group and 336 cases (129 males and 207 females) in the nonsarcopenia group. The average age and excess hs-CRP rate in the sarcopenia group were higher than those in the nonsarcopenia group, while the average BMI and the incidence of NAFLD were lower than those in the nonsarcopenia group (*P* < 0.05, Table 1). Sarcopenia and NAFLD were negatively correlated (Table 2).

In the logistic regression analysis, BMI, sex, age, high blood pressure, diabetes, HbA1c, high uric acid hematic disease, hs-CRP, levels of ALT, AST, TC, TG, LDL, and HDL, and NAFLD were independent variables, and sarcopenia was the dependent variable. Additionally, BMI was a protective factor for sarcopenia, while age, sex, and hs-CRP were risk factors for sarcopenia (*P* < 0.05, Table 3). NAFLD was neither a risk-factor nor a protective factor for sarcopenia.

4. Discussion

Sarcopenia is characterized by reduced muscle mass and decreased muscle function, both of which increase the risk of falls and reduce the ability of elderly individuals to live on their own. The rising prevalence of NAFLD has paralleled the obesity epidemic in western and developing countries, which will soon make NAFLD the most common liver disease worldwide [8]. Sarcopenia has been recognized as a new geriatric syndrome. The risk of NAFLD increases with age [9]. With increasing age, both reduced muscle mass and presence of NAFLD threaten the health of elderly populations.

TABLE 3: Logistic regression analysis of the association between sarcopenia and NAFLD, BMI, age, sex, and hs-CRP.

	Sarcopenia	OR	95% CI
BMI (<18.5)	78.26%	Control	
BMI (~18.5)	45.50%	0.185	0.123–0.279
BMI (≥24)	15.66%	0.0342	0.0151–0.0778
Age (<70)	25.79%	Control	
Age (~70)	28.57%	1.872	1.394–2.512
Age (≥80)	55.95%	3.504	1.943–6.310
Female	35.97%	Control	
Male	40.28%	2.417	1.553–3.762
hs-CRP (normal)	26.11%	Control	
hs-CRP (excess)	42.2%	2.283	1.459–3.573

$P < 0.05$.

Studies have explored the existence of an intrinsic correlation between the two conditions, and muscle loss was identified as a risk for NAFLD [10, 11]. NAFLD can be clearly diagnosed, combined with alcohol intake history, and ultrasound manifestation with good sensitivity and specificity. In this study, abdominal ultrasound was used to diagnose NAFLD.

In our study, the results of logistic multiple regression analysis showed that age, hs-CRP, sex, and BMI were associated with sarcopenia. Age is a risk factor for sarcopenia. The risk of suffering from sarcopenia in the oldest age group (≥80 years) is 3.504 times higher than that in younger age groups (<70 years) (odds ratio (OR) = 3.504; $P < 0.05$). At present, it is believed that chronic inflammation is one of the pathophysiological mechanisms underlying sarcopenia. Excess hs-CRP, with a 2.283 times higher risk of sarcopenia than normal values (OR = 2.283; $P < 0.05$), is also a risk factor for sarcopenia. In our study, men were 2.417 times more likely to suffer from sarcopenia than were women (OR = 2.417; $P < 0.05$). Androgens may play an important role in maintaining muscle mass. The decrease in androgen levels in men with increasing age may lead to an increased prevalence of sarcopenia. In addition, elderly women participate more in physical activity, such as housework, shopping, and square dancing, in their daily life than do men. It is known that exercise increases muscle mass. We also found that the incidence of sarcopenia decreased with increasing BMI. Compared with the group with BMI < 18.5 kg/m², there was a significant decrease in the risk of sarcopenia in the group with BMI ≥ 24 kg/m² (OR = 0.0342; $P < 0.05$). Muscle is one of the important components of body mass; thus, when muscle mass increases, BMI does as well. Therefore, BMI is a protective factor to sarcopenia.

Our conclusions are different from the Korean Sarcopenic Obesity Study [11]. Correlation analysis between NAFLD and sarcopenia showed that the two diseases are negatively correlated ($R = -0.15$; $P = 0.001$). NAFLD is neither a risk factor nor a protective factor for sarcopenia according to the results of our logistic multiple regression analysis. Additionally, the Korean study lacked measurement of muscle strength and muscle function and was not specifically designed for the elderly. The study showed that muscle loss was a risk factor to NAFLD, but it did not demonstrate the relationship

between sarcopenia and NAFLD. Low muscle mass and high fatty mass arise simultaneously in sarcopenic obesity. However, the prevalence of sarcopenic obesity in the elderly is approximately 5.8% [12]. In conclusion, previous studies on the relationship of sarcopenia and NAFLD need to be revised.

Nevertheless, the present cross-sectional study has some limitations. First, the selection of subjects may be affected by certain sampling errors. Second, it is possible that the coexistence of multiple diseases and long hospitalization may have influenced the final result. Therefore, the exact link between NAFLD and sarcopenia remains to be explored further.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Speculation of the Time-Dependent Change of FIB4 Index in Patients with Nonalcoholic Fatty Liver Disease: A Retrospective Study

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Aim. To speculate on the time-dependent change of FIB4 index in patients with nonalcoholic fatty liver disease (NAFLD) and its increase-decrease rate per year, simply and accurately. **Methods.** In all 23 patients with NAFLD with the value of FIB4 index over 1.30 at the peak, the period from the first to each examination date was calculated and this period (years) was regarded as x . Next, the mean value of FIB4 index during the past year to each examination date was regarded as y . In every y , the minimum and the maximum y value were found out. Between x corresponding to this minimum y and x corresponding to this maximum y , the correlation between x and y was analyzed as the main subject. Then, the slope of each correlation was investigated, because it should indicate increase-decrease rate per year. **Results.** In all 23 patients, the correlations as the main subject were recognized and the mean absolute value of correlation coefficient (r) was 0.91 ± 0.08 . As for the slope, the mean absolute value was 0.1371 ± 0.1147 (/year). **Conclusion.** Simply and accurately, the time-dependent change of FIB4 index and its increase-decrease rate per year could be approximately speculated.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide [1–5]. A liver biopsy still remains the gold standard for the diagnosis of nonalcoholic steatohepatitis (NASH), but it is difficult to perform liver biopsies in all patients with NAFLD. Therefore many noninvasive methods for estimating liver fibrosis have been developed; these are direct markers and the scoring systems, such as type IV collagen 7S [6, 7], hyaluronic acid [8, 9], aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR) [9, 10], NAFLD fibrosis score [11], BARD score [12], NAFIC score [7], and so on.

FIB4 index has been developed to predict liver fibrosis in patients with HIV/HCV coinfection [13] and it is also useful for estimating liver fibrosis in patients with NAFLD [14–16]. However there were few reports analyzing the transition of FIB4 index during all the clinical period in patients with NAFLD. Probably for the dispersion of the data, it has been difficult to estimate the accurate value.

In this study the correlation between the period from the first to each examination date and the mean value of FIB4 index during the past year to each examination date was analyzed. This correlation was thought to be the time-dependent change of the mean FIB4 index during the past one year and in the present study the correlation was proved to be extremely strong. Moreover, increase-decrease rate per year could be derived from the slope of the correlation in the scatter diagram.

In this retrospective study, the aim was to speculate approximately on the time-dependent change of FIB4 index and its increase-decrease rate per year, simply and accurately.

2. Methods

2.1. Patients. A total of 23 patients between October 1999 and June 2017 were enrolled with the following criteria: negative HBs antigen, negative HCV antibody, and negative anti-mitochondrial antibody [17]. Serum CRP levels were continuously negative. Patients whose values of anti-nuclear antibody

(ANA) showed more than 1 : 160 were excluded [18]. The peak ALT levels were over 40 (U/L) for males or over 30 (U/L) for females [19–21]. The peak value of FIB4 index was over 1.30 [14, 15, 22] in every patient. Fatty liver was diagnosed with ultrasonography and/or computed tomography. Drug induced liver injury and hereditary liver diseases were denied by the interview. Patients who consumed alcohol over 30 g per day for males or over 20 g per day for females were excluded [14, 23, 24]. Patients whose observed period in the clinic was less than two years were excluded. Finally patients whose maximum interval between examinations was more than one year were excluded.

All procedures in this study were conducted with the declaration of Helsinki (1964). The written informed consent was not applicable, because this is a retrospective study. In this study, direct data of AST, ALT, age, and platelet count were only used in patient characteristics and it was not possible to identify individuals.

2.2. Correlations

2.2.1. The Main Correlations. First, the period from the first to each examination date was calculated and this period (years) and was regarded as x . Next, the mean value of FIB4 index during the past one year to each examination date (the mean FIB4 index YTD) was regarded as y . Because of using the mean value during the past one year as y , x less than 1.00 (years) and y corresponding to this x were excluded; the minimum x value in every x was more than 1.00 (years). In every y , the minimum y value and the maximum y value were found out. Between x corresponding to this minimum y and x corresponding to this maximum y , the correlation between x and y was analyzed in every patient. This correlation was defined as the main correlation. There are two possibilities; either the values of correlation coefficient (r) are positive or these are negative. The group with positive value of r was defined as FIB4 index-increasing group and the group with negative value of r was also defined as FIB4 index-decreasing group.

2.2.2. The After-Main Correlations. Then, another correlation was analyzed, except for the data during the period of the main correlation. However, both ends of the data in the main correlation were not excluded.

After the period of the main correlation, it was checked whether the period to the last examination date was more than 1.00 (years) or not. Only when this period was more than one year, the analysis was performed. The first data of this analysis was automatically the last data in the main correlation. In FIB4 index-increasing group, the minimum y value was newly found out in this period. Yet, in FIB4 index-decreasing group, the maximum y value was newly found out. In both groups, from the maximum x in the main correlation to x corresponding to y newly found out, the correlation between x and y was analyzed. This correlation was defined as the after-main correlation.

2.2.3. The Before-Main Correlations. Finally, before the period of the main correlation, it was checked whether the

period from the first examination date was more than 2.00 (years) or not, because x less than 1.00 (years) had been excluded. Only when this period was more than two years, the analysis was performed. The last data of this analysis was automatically the first data in the main correlation. In FIB4 index-increasing group, the maximum y value was newly found out in this period. Yet in FIB4 index-decreasing group, the minimum y value was newly found out. In both groups, from x corresponding to y newly found out to the minimum x in the main correlation, the correlation between x and y was analyzed. This correlation was defined as the before-main correlation.

2.2.4. A Total of the Correlations Recognized in the Study. The cumulative correlations recognized in this study were shown.

2.3. Slopes of Correlations. In every patient, increase-decrease rate per year of the mean FIB4 index YTD was derived from the slope of the main correlation. In the same way it was also derived from each slope of the after-main correlation and/or the before-main correlation, if these correlations were recognized.

2.4. Statistics Analysis. Each correlation between two parameters was evaluated by Pearson's correlation. A p value (p) less than 0.05 was considered statistically significant. It was conducted by Microsoft Excel for MAC 2011.

3. Results

3.1. Patient Characteristics. 12 out of 23 patients (52.2%) were male. In 20 patients the values of ANA were less than 1 : 40 [18] and in three patients these were 1 : 40, 1 : 40, and 1 : 160, respectively, and the values of anti-smooth muscle antibody were all less than 1 : 40 and also immunoglobulin G levels were all within the upper normal limit of the clinic [25]. In 17 patients computed tomography scans were performed. In all patients, the mean value of the peak ALT levels was 72 ± 35 (U/L) and that of the peak value of FIB4 index was 2.84 ± 1.34 . In 10 patients the peak values of FIB4 index were more than 2.67 [14, 21], yet in nine patients those were less than 2.00. In all patients, the mean value of platelet count at the bottom was 165 ± 45 ($\times 10^9$ /L). Of 23 patients, 19 consumed no alcohol and the remaining four were all males (Table 1).

3.2. Correlations

3.2.1. The Main Correlations. In all 23 patients the main correlations were recognized (Figure 1) and the mean absolute value of r was 0.91 ± 0.08 (Table 2). Each p was shown in Table 2. Of 23 patients, 17 were categorized in FIB4 index-increasing group and the mean value of r was 0.90 ± 0.09 (0.69 to 0.99). In 11 of these 17, the values of r were more than 0.90. On the other hand, six of 23 patients were categorized in FIB4 index-decreasing group and the mean value of r was -0.94 ± 0.02 (-0.97 to -0.91). In all these six patients, the absolute values of r were more than 0.90. Therefore, in 17 out of 23 patients, the absolute values of r were more than 0.90. In a total of 23 patients, the mean value of interval between

TABLE 1: Characteristics of all 23 patients.

Patients (<i>n</i> = 23)	Laboratory findings		
	At the first examination	Peak value (bottom value only as for platelet count)	At the last examination
Gender (male)		12 (52.2%)	
Age (years)	58.2 ± 8.5	NA	68.8 ± 9.5
AST (U/L)	40 ± 26	57 ± 30	28 ± 10
ALT (U/L)	49 ± 35	72 ± 35	26 ± 12
GGT (U/L)	NA	94 ± 83	45 ± 44
FIB4 index	1.66 ± 0.78	2.84 ± 1.34	2.04 ± 0.82
AAR	0.88 ± 0.22	1.52 ± 0.34	1.17 ± 0.31
Platelet count (×10 ⁹ /L)	215 ± 68	165 ± 45	207 ± 63
Type IV collagen 7S (ng/mL)	NA	5.2 ± 2.0	4.4 ± 1.4
M2BPGi	NA	1.09 ± 0.86	0.88 ± 0.74
Mean ± SD			

Continuous variables were shown as mean ± standard deviation. At the last examination both type IV collagen 7S and M2BPGi were examined in all 23 patients. *n*, number of patients; NA, no analysis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; AAR, AST/ALT ratio; M2BPGi, Mac-2 binding protein.

examinations was 0.17 ± 0.09 (years), that is, 64 ± 33 (days), and the mean value of the total clinical period was 10.7 ± 4.6 (years) (Table 2). Since *x* less than 1.00 (years) were excluded, the total analyzed period was 9.5 ± 4.5 (years) (Table 2). The period in which the main correlation was recognized (the main correlation's period) was 6.6 ± 4.5 (years) and the mean ratio of the main correlation's period to the total analyzed period was 64 ± 23% (27% to 98%).

3.2.2. The After-Main Correlations. In 11 out of all 23 patients, each period to the last examination after the main correlation was more than 1.00 (years). In eight of these 11, the after-main correlations were seen. The mean absolute value of *r* was 0.93 ± 0.04 and each *p* was shown in Table 3. In the remaining three of these 11, that is, in patients of cases 5, 16, and 18, the correlations were not recognized statistically. In these three patients, numbers of analyzed data were four, five, and five, respectively, and the correlations were not recognized by *p* = 0.17 and *r* = -0.83, by *p* = 0.09 and *r* = -0.82, and by *p* = 0.07 and *r* = 0.85, respectively (Table 3).

3.2.3. The Before-Main Correlations. In 14 out of all 23 patients, each period from the first examination before the main correlation was more than 2.00 (years). In 10 of these 14, the before-main correlations were seen. The mean absolute value of *r* was 0.95 ± 0.05 and each *p* was shown in Table 3. In the remaining four of these 14, that is, in patients of cases 6, 8, 11, and 20, the correlations were not recognized statistically. In two patients of cases 6 and 11, numbers of analyzed data for the correlations were both two and it was impossible to analyze. In the remaining two patients of cases 8 and 20, numbers of analyzed data were three and four and the correlations were not recognized by *p* = 0.34 and *r* = -0.86 and by *p* = 0.27 and *r* = 0.73, respectively (Table 3).

3.2.4. A Total of the Correlations Recognized in the Study. The cumulative number of all correlations recognized in this

study was 41 (Table 3). The mean absolute value of *r* was 0.92 ± 0.07. In 32 of 41 correlations the absolute values of *r* were over 0.90 and in only three of 41 they were less than 0.80 (0.688 to 0.799).

3.3. Slopes of Correlations. In all 23 main correlations, the values of increase-decrease rate per year of the mean FIB4 index YTD were shown as the slope in Table 2. In them the mean absolute value of the slope was 0.1371 ± 0.1147 (/year). In 17 correlations categorized in FIB4 index-increasing group, the mean value of the slope was 0.1212 ± 0.1114 (/year), yet in six ones categorized in FIB4 index-decreasing group, it was -0.1823 ± 0.1117 (/year). Then, in a total of 41 correlations, the mean absolute value of the slope was 0.1764 ± 0.1307 (/year). In 22 positive correlations, the mean value of the slope was 0.1415 ± 0.1118 (/year), yet in 19 negative correlations, it was -0.2168 ± 0.1319 (/year). All 41 correlations were shown in Figure 2. In order to demonstrate the slopes clearly, the main correlations were shown without *y*-intercept in Figure 2.

4. Discussion

In the present study the correlations between the period from the first to each examination and the mean FIB4 index YTD were analyzed. The results just would mean the time-dependent change of the mean FIB4 index YTD. All 23 enrolled patients had at least one phase with the main correlation (Figure 1 and Table 2) and the mean absolute value of *r* was 0.91 ± 0.08. In 17 of these 23 (74%) the absolute values of *r* were over 0.90. Meanwhile, 10 of 23 patients had only one phase with the main correlation (Figure 2(a)) and the remaining 13 had several phases (Figures 2(b)–2(d)). As a result, a total of 41 correlations were recognized in the study and the mean absolute value of *r* was 0.92 ± 0.07. In 32 of all the 41 correlations (78%) the absolute values of *r* were over 0.90 (Table 3). In addition, the authors will show the reason why there were some correlations with low absolute values

TABLE 2: All 23 main correlations.

Patients Case number	Slope		<i>P</i>	<i>r</i>		<i>r</i> ²	Interval between examinations [#]		The main correlations period (years)	Ratio (%)	Total analyzed period (years)	Total clinical period (years)
	Positive value	Negative value		Positive value	Negative value		(years)	(days)				
Case 1	0.4098		2×10^{-51}	0.98		0.96	0.12	44	8.2	64	12.8	13.9
Case 2	0.3343		1×10^{-20}	0.98		0.96	0.08	29	2.4	28	8.7	9.7
Case 3	0.2698		5×10^{-33}	0.96		0.92	0.18	66	9.5	94	10.1	11.1
Case 4	0.1923		9×10^{-8}	0.99		0.98	0.12	44	1.2	72	1.6	2.7
Case 5	0.1618		0.01	0.96		0.92	0.19	70	1.1	32	3.3	4.4
Case 6	0.084		2×10^{-8}	0.93		0.87	0.43	156	8.8	73	12.1	13.6
Case 7	0.0762		9×10^{-7}	0.82		0.67	0.19	71	3.4	35	9.6	10.9
Case 8	0.0758		1×10^{-42}	0.92		0.85	0.16	58	14.7	93	15.9	17.3
Case 9	0.0755		3×10^{-44}	0.91		0.83	0.09	35	9.9	63	15.8	17.1
Case 10	0.0708		2×10^{-53}	0.94		0.88	0.11	42	12	98	12.2	13.5
Case 11	0.0652		4×10^{-43}	0.96		0.91	0.15	54	9.4	84	11.2	12.5
Case 12	0.0629		1×10^{-32}	0.84		0.71	0.13	47	14.1	96	14.8	16
Case 13	0.0608		6×10^{-10}	0.8		0.64	0.27	97	9.1	80	11.4	12.5
Case 14	0.0349		5×10^{-8}	0.69		0.47	0.1	38	4.8	67	7.1	8.2
Case 15	0.0302		1×10^{-13}	0.74		0.55	0.2	72	13.9	98	14.2	15.3
Case 16	0.0299		6×10^{-11}	0.89		0.79	0.29	105	7.6	75	10.1	11.2
Case 17	0.0256		6×10^{-48}	0.95		0.9	0.11	40	9.5	61	15.5	16.6
Case 18		-0.089	0.004		-0.95	0.9	0.21	76	1.2	53	2.3	3.5
Case 19		-0.0984	5×10^{-12}		-0.91	0.82	0.11	39	2.9	43	6.8	7.9
Case 20		-0.1078	0.005		-0.95	0.89	0.4	145	2	47	4.2	5.2
Case 21		-0.1237	6×10^{-5}		-0.94	0.88	0.13	46	1.3	63	2.1	3.1
Case 22		-0.307	2×10^{-11}		-0.97	0.93	0.12	44	2	37	5.5	6.6
Case 23		-0.3681	2×10^{-11}		-0.95	0.9	0.12	45	3.2	27	11.9	12.9
Mean \pm SD	0.1212 \pm 0.1114	-0.1823 \pm 0.1117	0.1371 \pm 0.1147	0.90 \pm 0.09	-0.94 \pm 0.02	0.83 \pm 0.13	0.17 \pm 0.09	64 \pm 33	6.6 \pm 4.5	64 \pm 23	9.5 \pm 4.5	10.7 \pm 4.6

[#] Each value was shown in total clinical period; continuous variables were shown as mean \pm standard deviation. Slope, the slope of the correlation; *P*, a *P* value; *r*, correlation coefficient; *r*², a squared value of *r*; The main correlation's period, the period in which the main correlation was recognized; Ratio, the ratio of the main correlation's period to the total analyzed period; Total analyzed period, the period from the earliest examination date at least a year after the first examination to the last examination date; Total clinical period, the period from the first to the last examination date.

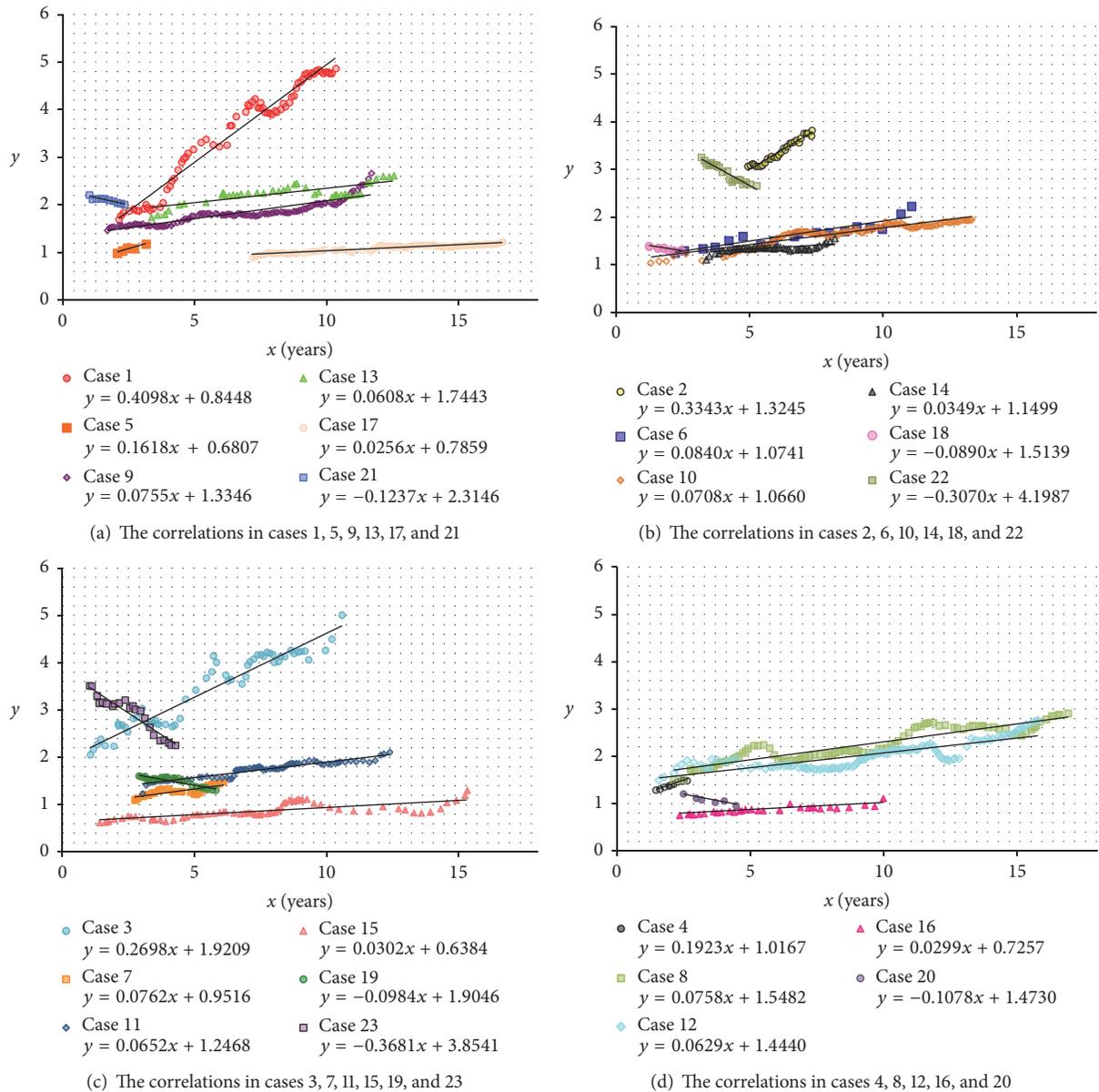


FIGURE 1: The correlation between the period from the first examination to each examination (x) and the mean value of FIB4 index during the past year to each date of examination (y) in the phase of all 23 main correlations.

of r . For example, out of all 41, three correlations (7.3%) with the absolute values of r less than 0.80 were recognized and all of them were the main correlations. They were enumerated in Figure 3. In all the three correlations the mean FIB4 index YTD gradually increased and then at once reached the peak, which was so-called “second peak point.” After this point to the last point of data in the correlation, there was the bottom point, which was so-called “second bottom point.” In these correlations the mean FIB4 index YTD moved like an italic type of “N.” In such a condition, the absolute value of r would probably become low.

In statistics, the coefficient of determination, which is calculated as a squared value of r (r^2), determines how enough the outcomes could be explained by the hypotheses.

In this study, the mean value of r^2 in the main correlation was 0.83 ± 0.13 and that in a total of 41 correlations was 0.86 ± 0.12 . The value of r^2 like these could not be ignored, even if the number was 23 or 41. Moreover, it had been explained why there were some correlations with low absolute values of r . Statistically it was thought to be sufficient to speculate how the mean FIB4 index YTD moved.

There was another important thing about the movement of the mean FIB4 index YTD. In three of five patients with three phases (Figure 2(b)), the mean FIB4 index YTD showed decreasing firstly, increasing secondly, and decreasing finally, yet in two of these five (Figure 2(b)), it showed increasing, decreasing, and increasing. On the other hand, in eight patients with two phases (Figures 2(c)-2(d)), it showed firstly

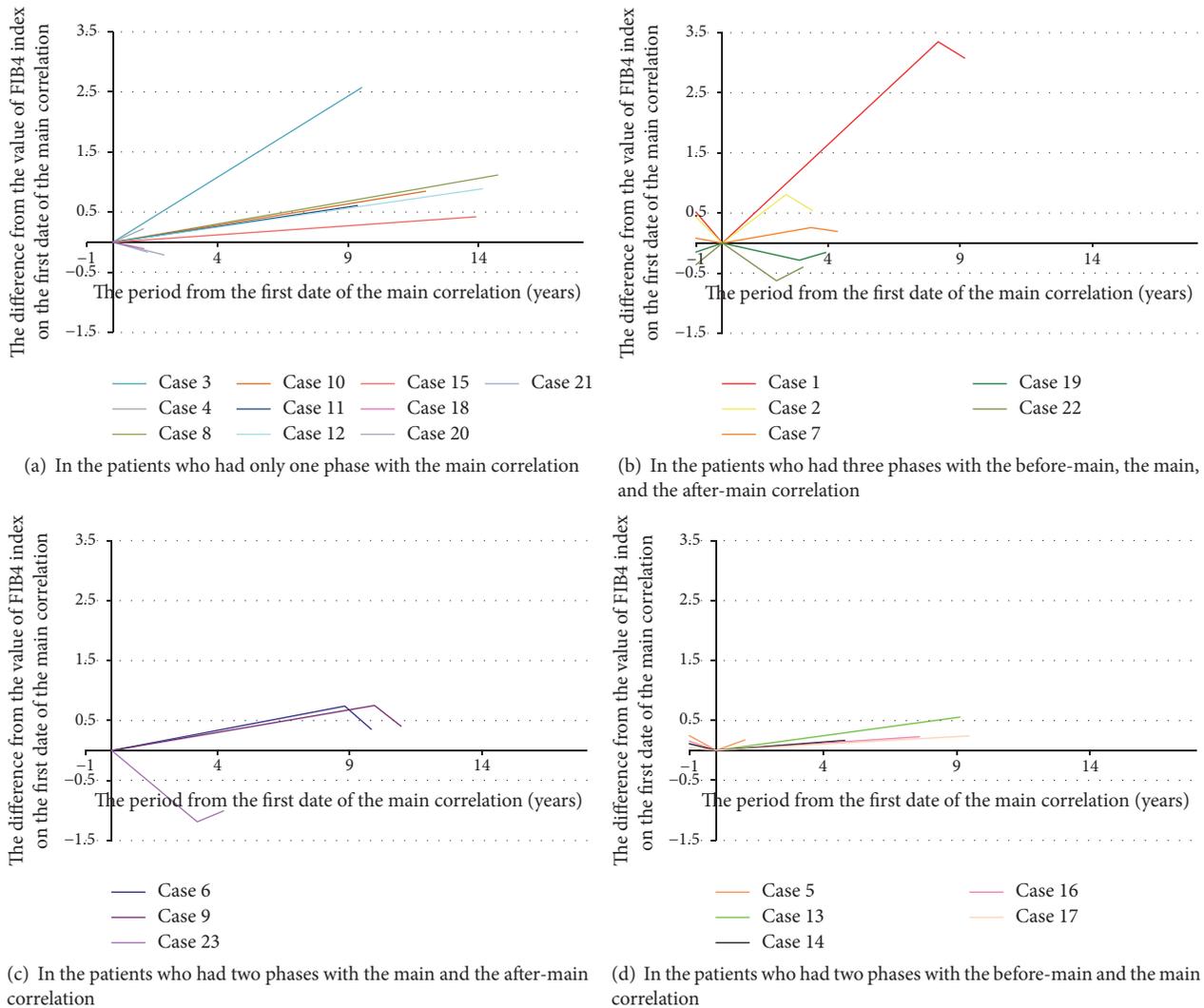


FIGURE 2: The slopes of all 41 correlations recognized in this study. All the main correlations were shown without y-intercept. (b, c, d): the before-main and the after-main correlations were shown only for a year.

decreasing and finally increasing or firstly increasing and finally decreasing. From the viewpoint of the movement, the most important thing was that there was a turning point in which the mean FIB4 index YTD changed from increasing to decreasing or from decreasing to increasing. This means that the mean FIB4 index YTD moved like a wave. Even in the main correlations, these waves were seen and the typical ones had been picked up in Figure 3.

Now, developing this study's methods, there would be a possibility. The possibility is that the methods will be applicable for any partial period. In order to validate it, the analysis only has to be performed, not from the first examination date and/or not to the last examination date. For example, in all the 23 patients the period was newly set from the closest date after half the total clinical period to that date after three-quarters. Limiting to this period, the analysis was newly performed through this study's methods. Out of 23 patients, seven whose analyzed period remained less than two years were excluded, because it was necessary for a year to

calculate the mean FIB4 index YTD. In all the remaining 16 out of 23, the new main correlations were analyzed. In the patient of case 16 there were only two pieces of data and it was impossible to analyze the correlation and in the patient of case 13 there were three pieces of data and the correlation was not statistically recognized by $p = 0.08$ and $r = 0.99$. However, in the remaining 14 patients the new main correlations were recognized and the mean absolute value of r was 0.92 ± 0.05 . In addition, in all 14, the absolute values of r were over 0.80. From this result, it was thought that the methods might be applicable for any partial period (Table 4).

The trigger of the start of the present study was a case report of a patient with NAFLD that the authors have already reported previously [26]. In this report we analyzed the correlation in a partial period between time (years) and the direct data of FIB4 index and we showed in that period that the direct data of FIB4 index decreased with the rate of 0.15 per year, statistically proven by the general linear regression model; $\text{FIB4 index} = 4.90 - 0.15 \times \text{time (years)}$ ($p = 0.02$).

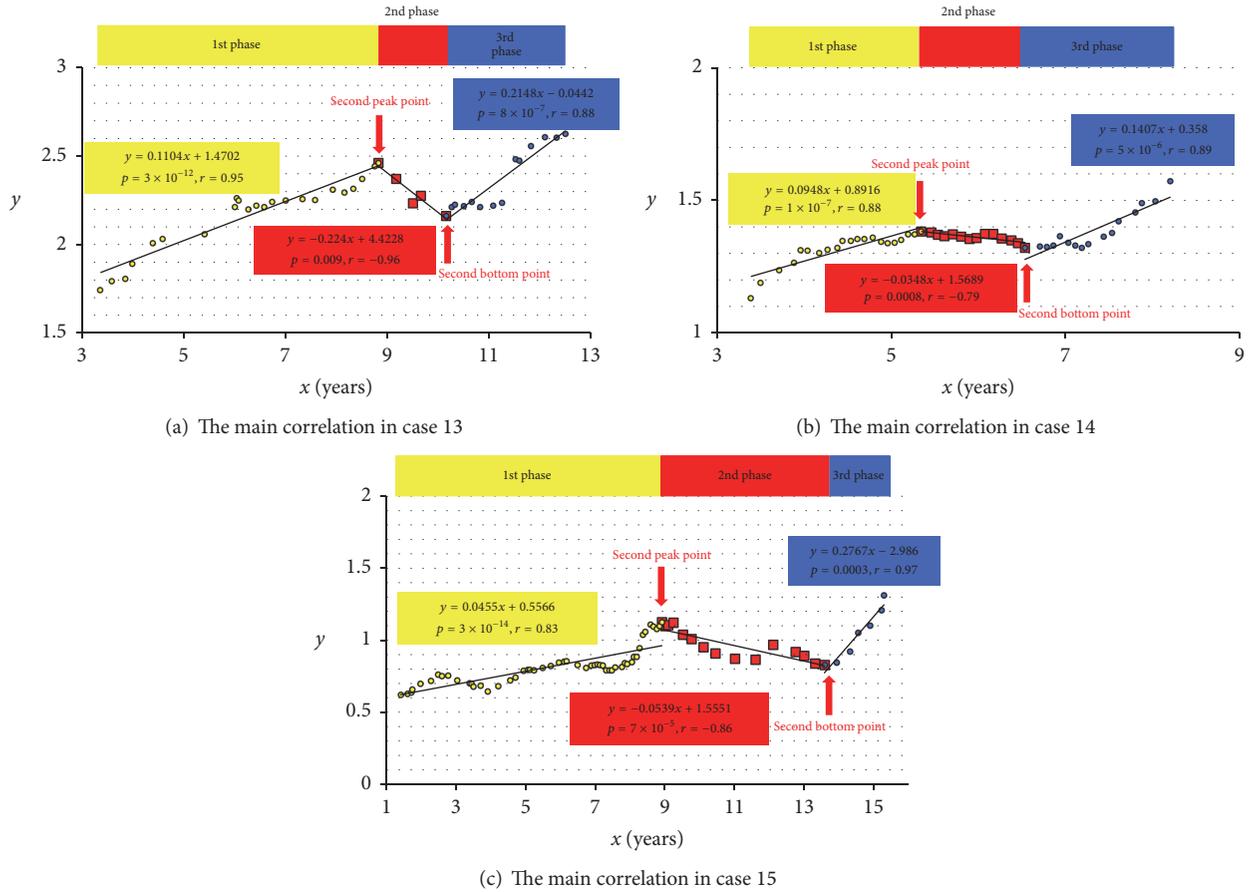


FIGURE 3: All the three correlations with the absolute value of r less than 0.80. The value of x was defined as the period from the first to each examination date and also the value of y was defined as the mean value of FIB4 index during the past one year to each examination date. In all the three correlations, values of y gradually increased and then at once reached the peak, which was so-called “second peak point.” After this point to the last point of data in each correlation, there was the bottom point, which was so-called “second bottom point.”

TABLE 4: All 14 new main correlations analyzed for the period newly set.

Case number of the patient	Slope	Absolute value of slope	p	Absolute value of r	n
Case 1	0.415	0.415	6×10^{-10}	0.9	25
Case 2	0.4296	0.4296	9×10^{-8}	0.97	13
Case 3	0.6245	0.6245	0.001	0.89	9
Case 6	0.1186	0.1186	0.045	0.82	6
Case 7	-0.1015	0.1015	0.035	0.9	5
Case 8	0.3461	0.3461	4×10^{-11}	0.99	13
Case 9	0.4383	0.4383	1×10^{-12}	0.99	16
Case 10	0.0921	0.0921	3×10^{-9}	0.89	25
Case 11	0.1192	0.1192	1×10^{-10}	0.96	19
Case 12	0.1062	0.1062	5×10^{-16}	0.95	31
Case 14	-0.0458	0.0458	0.004	0.91	7
Case 15	-0.1094	0.1094	8×10^{-6}	0.96	10
Case 17	0.1304	0.1304	0.004	0.88	8
Case 23	0.3707	0.3707	0.004	0.85	9
The mean \pm SD		0.2462 ± 0.1766		0.92 ± 0.05	

Continuous variables were shown as mean \pm standard deviation. In all the 23 patients the period was newly set from the closest date after half the total clinical period to that date after three-quarters. Of 23 patients, seven whose analyzed period remained less than two years were excluded. In the remaining 16 patients, the new main correlations were analyzed. In two of these 16, the correlations were not recognized. In case 16 the correlation could not be analyzed because of only two data and in case 13 number of data was three and the correlation was not recognized by $p = 0.08$ and $r = 0.99$. Slope, the slope of the correlation; p , a p value; r , correlation coefficient; n , number of data to analyze the correlation.

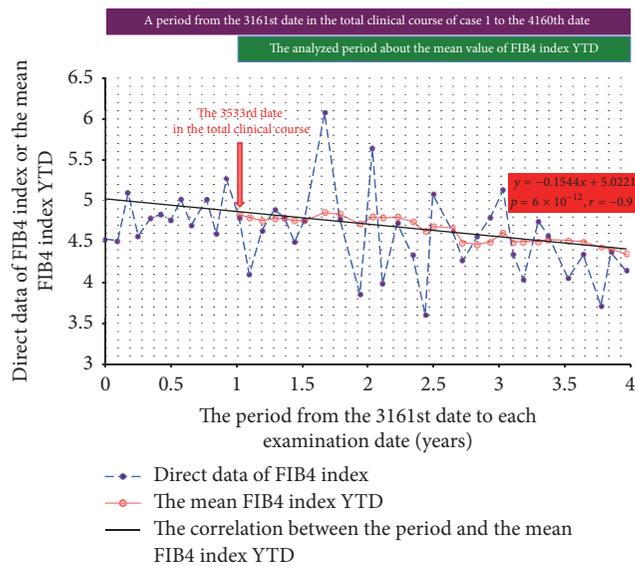


FIGURE 4: The transition of FIB4 index in a partial period in case 1. In a patient of case 1, a correlation was analyzed in a partial period newly set. The period was set from the 3161st date in the total clinical course to the 4610th date. After the earliest date more than a year from the 3161st date, that is, the 3533rd date finally to the 4160th date, the correlation between the period (years) from the 3161st date and the mean FIB4 index YTD was analyzed through this study's method. All the data were cited from our previous case report [26]. The mean FIB4 index YTD, the mean value of FIB4 index during the past one year.

This patient was enrolled as a patient of case 1 in the present study. If this study's methods were applicable for any partial period, similar outcome should be obtained. As expected, the mean FIB4 index YTD decreased with the rate of 0.15 per year (Figure 4); the mean FIB4 index YTD = $5.02 - 0.15 \times \text{time}$ (years) ($p = 6 \times 10^{-12}$ and $r = -0.91$). This was very similar to our previous formula and p has become extremely low.

In this way, it was very easy to estimate increase-decrease rate per year of the mean FIB4 index YTD. It was the value of the slope of each correlation on the scatter diagram. As for the main correlations, the mean absolute rate per year was 0.1371 ± 0.1147 (Table 2). In this viewpoint, since the difference of FIB4 index between 1.30 and 2.67 [14, 27] is 1.37, it would take about 10 years by the mean absolute rate per year.

Meanwhile, the limitations of this study should be shown. Firstly, interval between examinations in this study was 0.17 ± 0.09 (years) and it would be rather short. If the interval was longer, the dispersion of the data could not be minimized and the strong correlation might not be recognized. In fact, the after-main correlations or the before-main correlations were not recognized statistically in some patients, probably for the lack of number of data. About this, if the interval in the correlations had been shorter, the number of data would have increased and the correlation might have been seen. Anyway, when there were at least six pieces of data for the analysis, the correlations were all recognized in this study.

Secondly, it took a year from the first examination to calculate the data and also took another year to analyze the data.

Thirdly, in this study histological findings were not performed. Certainly, it was speculated that, compared to the earlier studies, liver fibrosis in the patients of this study would be rather advanced. Several proofs should be shown as follows. In this study the mean value of FIB4 index at the peak was 2.84 ± 1.34 (Table 1). Shah et al. reported that, for advanced fibrosis (stage 3-4), a FIB4 > or = 2.67 had an 80% positive predictive value [14]. Moreover, the mean value of platelet count at the bottom was 165 ± 45 ($\times 10^9/L$) (Table 1). Kaneda et al. reported that the platelet count was found to be an independent predictor of cirrhosis and a cut-off value of $16 \times 10^4/\text{microL}$ for the platelet count was associated with an optimal combination of sensitivity (100%) and specificity (95%) [8]. In addition, the mean value of type IV collagen 7S at the peak was 5.2 ± 2.0 (ng/mL) (Table 1). It was reported that in patients with NASH the type IV collagen 7S domain was significantly elevated in patients with advanced fibrosis by multiple regression analysis [6] and Sumida et al. have developed NAFIC score in biopsy-proven patients with NAFLD to differentiate NASH from NAFLD, using the cut-off of type IV collagen 7S ≥ 5.0 (ng/mL) [7]. Then, the mean value of M2BPGi at the peak was 1.09 ± 0.86 (Table 1). Nishikawa et al. reported that in NASH patients the median values in each fibrosis stage were 0.7 COI in F1, 0.7 COI in F2, 1.2 COI in F3, and 2.4 COI in F4 [28] and Lai et al. also reported that the AUROC of the COI for the diagnosis of fibrosis stages ≥ 1 , ≥ 2 , ≥ 3 , and 4 was 0.61, 0.71, 0.74, and 0.84, respectively [29]. These facts would show liver fibrosis in the enrolled patients would be rather advanced.

However, it is a problem whether the time-dependent change of FIB4 index corresponds to that of fibrosis by a liver biopsy. About this there was a retrospective study. McPherson et al. reported the following [30]. In 108 patients who had serial biopsies (median interval 6.6 years, range 1.3–22.6), there was a significant relationship between the change in fibrosis between biopsies and the change in both NAFLD fibrosis score [11] and FIB-4 score. They compared patients with histological evidence of increasing fibrosis stage (progressors) to subjects whose fibrosis remained stable or regressed (nonprogressors) and in progressors FIB-4 score was changed from 1.85 ± 1.31 at baseline biopsy to 2.33 ± 1.69 at follow-up one, yet in nonprogressors it was changed from 1.26 ± 0.57 to 1.36 ± 0.62 [30].

Nevertheless, there were few studies about the relationship between change in FIB4 index and change in fibrosis conducted by paired biopsies and therefore further verifications should be done. However, in the process of verifying it, this study's methods might be useful, because the dispersion of a single direct data of FIB4 index probably would be a considerable problem. It is difficult to perform biopsies to all patients with NAFLD and in such a condition the methods to minimize the dispersion of the data would be helpful.

To consider the risk of liver fibrosis based on grasping the whole picture of the movement of the mean FIB4 index YTD would be one of the practical benefits in the study. For all practical purposes, the latest correlation means either

the main correlation or the after-main correlation and if the value of the slope of that correlation were positive, the progression of liver fibrosis would be concerned. Especially in a patient whose last data of the mean FIB4 index YTD in that correlation shows a value over 2.67, advanced liver fibrosis should be well considered. On the other hand, in a patient with a negative value of the slope of the latest correlation, even if the last data of the mean FIB4 index YTD shows a value over 2.67, it would be a little difficult to assess the risk. In fact, in a patient of case 1, a severe complication was gradually improved in such a condition [26]. However, in such a patient careful treatment should be done to prevent the progression of liver fibrosis. Finally, if the value of the slope is negative and also the last data of the mean FIB4 index YTD is less than 1.30 in the latest correlation, the risk of liver fibrosis is considered to be low.

We hope that the methods in this study will be the benefits to patients with NAFLD and in the future the methods will be compared to other markers and modalities for estimating liver fibrosis, with increased number of patients.

5. Conclusion

This study demonstrated that in patients with NAFLD the correlations between the period from the first to each examination date and the mean value of FIB4 index during the past one year to each examination date were strongly recognized. Approximately, the time-dependent change of FIB4 index and its increase-decrease rate per year could be speculated simply and accurately.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Cognitive Changes and Brain Volume Reduction in Patients with Nonalcoholic Fatty Liver Disease

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Studies of psychological condition of patients suffering from nonalcoholic fatty liver disease are rather equivocal about the results: while some claim that NAFLD patients suffer from anxiety and depression more than non-NAFLD controls, others do not withstand those findings. Lower cognitive potentials have also been reported, both in patient related and in animal model-based investigations, and correlated with assessed brain tissue changes. We hypothesized that NAFLD, as a condition, affects the brain tissue and, subsequently, the cognitive state. So we compared findings in 40 NAFLD positive and 36 NAFLD negative patients and correlated their brain tissue volumes with the results of Montreal Cognitive Assessment (MoCA) test. Binomial logistic regression verified the influence of NAFLD state leading to lower cognitive potentials: odds ratio 0.096; 95% confidence interval (CI) 0.032–0.289; $p < 0.001$. Patients with NAFLD had a greater risk to suffer from the cognitive impairment and depression: RR = 3.9; 95% CI 1.815–8.381; $p = 0.0005$ and RR = 1.65; 95% CI 1.16–2.36; $p = 0.006$. NAFLD significantly influenced the cognitive deficit and tissue volume reduction and patients suffering from NAFLD had about four times higher risk of having a cognitive impairment.

1. Introduction

Psychological condition of patients with nonalcoholic fatty liver disease (NAFLD) still remains rather ambiguous. Studies related to the mentioned problem are dealing mostly with depression, but results obtained are equivocal: some studies confirmed the presence of depression in correlation with histological severity of NAFLD [1–4], while other publications denied the relationship between them [5, 6]. A systematic review by Macavei and coworkers (2016) [7] also emphasized the presence of depression and anxiety as the most frequent mood disturbances in NAFLD suffering patients. Petta and coworkers (2016) [8] outlined the relationship between decreased volume of brain white matter, lower cognitive potentials, and the type of fibrosis level in NAFLD. In both the entire cohort and NAFLD cases, only females older than 45 and with F2–F4 fibrosis level

were independently associated with white matter lesions, mostly of mild grade. Some decrease in the blood oxygen concentration was revealed in female NAFLD patients and it was connected to lower cognitive performance in females affected by NAFLD [9].

The experimental investigation of NAFLD performed on Sprague-Dawley rats delineated the changes on hippocampal-related memory, but the deficit in hippocampal insulin signaling or brain-derived neurotrophic and insulin-like growth factor 1 appeared to have no influence on this [10].

We aimed to assess whether NAFLD influences the cognitive status in patients and if cognitive impairment correlates with lower volumes of various brain structures, including total brain white and gray matter volumes and volumes of the lateral ventricles in NAFLD patients.

TABLE 1: Demographic characteristics of examined cohort.

	Examined (40)	Control (36)	Difference and significance			
Age (yrs., mean \pm SD)	47.88 \pm 6.07	47.07 \pm 6.68	NS			
Sex (male/female)	22/18	16/14	NS			
Body mass index (BMI, kg/m ² , mean \pm SD)	32.04 \pm 6.67	22.18 \pm 4.43	$p < 0.001$			
Diabetes type 2 (yes/no)	12/28	9/27	NS			
Hypertension (yes/no)	32/8	16/20	Spearman correlation NAFLD + and hypertension, $p < 0.0001$			
HDL/LDL ratio (mean \pm SE)	4.49 \pm 0.23	2.94 \pm 0.18	$p < 0.0001$			
Status according to BMI:						
Normal	5	21				
Overweight	15	11	$p = 0.000028$			
Obese	20	4				
Metabolic syndrome (yes/no)	34/6	8/28	Chi square, $p < 0.00001$			
MoCA score (mean \pm SD)	24.07 \pm 3.18	27.17 \pm 2.35	$p < 0.001$			
Total brain volume (cm ³ , mean \pm SD)	1388 \pm 127.98	1417 \pm 191.83	NS			
Gray matter volume (gmw in cm ³ mean \pm SD)	405 \pm 37.42	414 \pm 56.09	NS			
White matter volume (wmw, in cm ³ mean \pm SD)	338 \pm 31.21	344 \pm 46.83	NS			
Lateral ventricle volume, right/left (lvlr, lvlr, in cm ³ mean \pm SD)	5.97 \pm 0.61	6.07 \pm 0.62	5.64 \pm 0.37	5.63 \pm 0.37	$p = 0.009^*$	$p < 0.001^*$
AST (U/l, mean \pm SE)	39.97 \pm 2.61	24.03 \pm 3.26	$p < 0.001$			
ALT (U/l mean \pm SE)	39.11 \pm 2.86	36.71 \pm 3.5	NS			
AST/ALT ratio (mean \pm SE)	1.13 \pm 0.11	0.7 \pm 0.06	$p = 0.01$			
C-reactive protein (mg/l, mean \pm SE)	3.96 \pm 0.45	3.52 \pm 0.51	NS			
Fasting glycaemia (mmol/l, l mean \pm SE)	5.8 \pm 0.19	3.71 \pm 0.25	$p < 0.001$			
Triglycerides (mmol/l, l mean \pm SE)	2.87 \pm 0.18	1.71 \pm 0.17	$p < 0.001$			

NS, not significant; SE, standard error. *Lateral ventricle volumes.

2. Material and Methods

The study group involved 89 first diagnosed, therapy naive patients, with high level of aminotransferases, out of which 40 (22 men and 18 women) aged 34–57 (mean 47.88 \pm 6.07) satisfied recruiting criteria, who were treated in the Department of Gastroenterology and Hepatology, or referred to the Gastroenterology outpatients service. Control group contained 30 patients, 16 men and 14 women, aged 39–53 (mean and standard deviation = 47.07 \pm 6.68) who were referred to the outpatient service for other gastroenterological problems, mostly of the functional origin (functional dyspepsia and irritable bowel syndrome), that do not involve liver nor gall bladder and gall pathways pathology, which were excluded by the abdominal ultrasonography. The demographic data are presented on Table 1.

Recruiting criteria are as follows:

- (1) Older than 18
- (2) No previous history of viral hepatitis of any kind, haemochromatosis, autoimmune hepatitis, cirrhosis, or other chronic liver disease
- (3) No presence of severe cardiopulmonary disease
- (4) Absence of obstructive sleep apnea syndrome assessed by polysomnography
- (5) The absence of endocrinological disorders: hypothyroidism, hypercorticism, syndrome of the polycystic ovaria
- (6) No history or clinical signs of excessive alcohol abuse (>20 g/day for males and >10 g/day for females)
- (7) No psychiatric disease and/or psychiatric medication history or any other hepatotoxic drugs
- (8) No visible traces of the illicit drugs abuse: negative urine multiple drug test on 10 kinds of drugs: cannabinoids, opiates, amphetamines, 3,4-methylenedioxymethamphetamine, cocaine/crack, benzodiazepines, tricyclic antidepressants, barbiturates, methadone, and buprenorphine
- (9) No visible focal or diffuse changes in the gray matter of the brain on MRI
- (10) Fazekas score 0 on MRI scan: Fazekas score is the estimated level of the white matter vascular changes and is the aftermath of brain vessels atherosclerotic changes

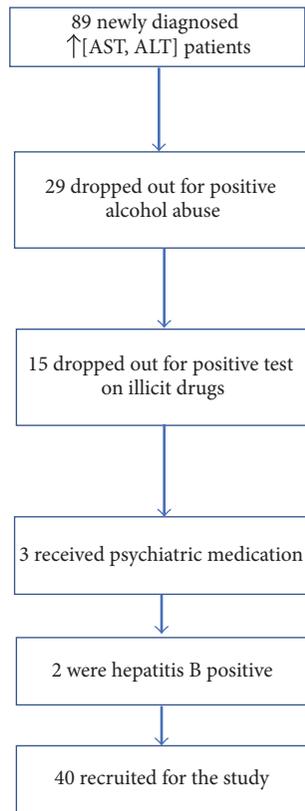


FIGURE 1: Flow diagram of patient selection from Department of Gastroenterohepatology.

- (11) Absence of any rheumatological disease
- (12) Patients who used antidiabetic drugs, insulin, antilipemic drugs, uricosuric drugs, steroids, and oral contraceptives were omitted from the study.

Most of the patients who were dropped out fell on the alcohol (29) and drug abuse tests (15). Flow diagram is shown on Figure 1.

Upon admittance, all the patients underwent abdominal ultrasonography and MRI brain scanning.

Abdominal ultrasound was performed using a 3.5 MHz transducer, by an experienced gastroenterologist. Both sub-costal and intercostal scannings were done. Images were captured in a standard fashion with the subject in the supine position and with the right arm raised above the head. In our clinical study we measured the following ultrasonographic parameters: anterior-posterior diameter of the liver (AP), homogeneity, echogenicity of the hepatic parenchyma, and the pancreas. Normal liver parenchyma was seen as solid homogenous echo texture, which was midway between the renal cortex and pancreatic echogenicity. Fatty liver disease was diagnosed as diffusely increased echogenicity of the hepatic parenchyma compared to the kidneys, vascular blurring, and deep-echo attenuation.

Sonographic evaluation (US) of hepatic steatosis was based on five criteria: parenchymal brightness, liver to kidney contrast, deep beam attenuation, bright vessel walls, and gallbladder wall definition [11, 12]. Diffuse hepatic steatosis

was graded I to III: I, increased hepatic echogenicity with visible periportal and diaphragmatic echogenicity; II, increased hepatic echogenicity with imperceptible periportal echogenicity, without obscuration of the diaphragm; and III, increased hepatic echogenicity with imperceptible periportal echogenicity and obscuration of the diaphragm.

Blood samples were collected after 12 hours of fasting. Analyses included platelet count (PC), platelet indices (MPV, PCT, and PDW), liver function tests, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lipid profile including triglycerides, C-reactive protein (CRP), and fasting blood glycaemia and insulin levels, by using Ilab 650 (Instrumentation Laboratory, Milano, Italy).

Volume measurements of the gray and white matter and lateral ventricles of the brain were performed on 3D T1-weighted MR images (Phillips Inc., Holland); acquisition parameters were as follows: TR = 9.8 ms; TE = 4.6 ms; flip angle = 8; section thickness = 1.2 mm; number of sections = 120; no section gap; whole brain coverage; FOV = 224 mm; matrix = 192; reconstruction matrix = 256. Routine T2-weighted and FLAIR images were performed to rule out a mass lesion as a contributory factor to memory loss or cognitive decline. The structures were manually outlined and compared with automatic extraction of the regions of interest in commercially available software. The software finally computed the volumes required.

After the diagnostic procedures, all the subjects underwent psychological testing for cognitive impairments using Montreal Cognitive Assessment test, Serbian version [13]. Test has several levels of testing, alternating connections (connect Figure 1 with letter A and then A to 2, to B, etc.), visuoconstructive abilities (draw a cube and a clock in 11:10 position of clock hands), memory (numbers repeated in the same and reverse order), attention (tap whenever you hear a letter A), serial subtraction of 7, starting with a hundred ($100 - 7 = 93$, $93 - 7 = 86$, etc.), sentence repeating, and verbal fluency. Maximal score is 30, 26 being the threshold for normal cognitive functioning.

Level of the depression was tested by Hamilton's depression rating scale of 21 items. Only first 17 items were scored: equal to or lower than 7, no depression; 8–13, mild depression; 14–18, moderate; 19–22, severe; equal to or higher than 23, very severe. For all the patients with depression diagnosis, a psychiatrist was consulted, and the subsequent therapy was individually prescribed. According to the protocol, patients were controlled every three months. The protocol involved liver examination by ultrasound, laboratory parameters, and psychiatric evaluation.

Statistical testing was performed by the commercially available software. Besides the measures of central tendency (mean and standard deviation (SD), minimum, and maximum), potential differences of mean values were assessed with one way analysis of variance (ANOVA) with Bonferroni post hoc correction, Student's *t*-test for independent samples for parametric, and chi square test for nonparametric data. Correlation was tested with Spearman and Pierson's correlation test. Possible dependence between the nonparametric

TABLE 2: Pierson's correlation of MoCA score values and observed volumes.

Volume	B	Beta	Significance
Total brain volume	0.025	1.5	NS
latvl	-39.18	-7.48	$p = 0.005$
latvr	-38.01	-7.46	$p = 0.007$
gmw	0.019	0.279	$p = 0.015$
wmw	0.2	2.89	$p = 0.009$

Latvl: lateral ventricle volume, left hemisphere; latvr: lateral ventricle volume, right hemisphere; gmw: gray matter volume; wmw: white matter volume.

parameters was estimated by binomial logistic regression. All the testing was performed on 95% probability level.

All the participants were acquainted in detail with the study aim and design before entering the program. They all signed a written consent afterwards.

3. Results

The cognitive status, according to MoCA index, was lower in NAFLD patients (Table 1). If we observe the cutoff value (26 points) and divide both groups into normal and subnormal groups, 26 patients in NAFLD group had lower MoCA score versus 6 control members (chi square = 19.23, $df = 1$, and $p < 0.0001$). Binomial logistic regression estimated significant influence of NAFLD on the reduced MoCA score: odds ratio 0.096, 95% confidence interval (CI) 0.032–0.289, and $p < 0.001$. Patients with NAFLD had a greater risk to suffer from cognitive impairment: RR = 3.9, 95% CI 1.815–8.381, and $p = 0.0005$, respectively.

Despite the fact that we did not evaluate differences in total brain, gray, and white volumes between the observed and control group, in NAFLD patients with lower MoCA score, the volumes of brain, gray, and white matter were significantly reduced: for brain volume, $t = 2.71$, $df = 38$, and $p = 0.01$, for gray matter volume, $t = 2.71$, $df = 38$, and $p = 0.01$ (absolutely correlated with brain volume), and for white matter, $t = 2.31$, $df = 38$, and $p = 0.026$. Furthermore, MoCA score values correlated with gray and white matter volumes, but not with total brain volume in NAFLD group. The increase in lateral ventricles volumes negatively correlated with total MoCA score (Table 2).

Difficulties appeared in NAFLD + group with questions that required postponed memory test to repeat the words after 5 minutes. Although no significant difference was obtained by chi square (chi = 3.5978, $p = 0.0057$), 22 examinees versus 12 controls did not solve the task. With question requiring attention, 28 examinees had more than two mistakes in tapping with pencil every time they heard the letter A, while the same mistake was revealed in controls in 9 cases (chi = 15.35, $p = 0.00009$).

Classified by Hamilton's depression score, there are more patients with moderate and severe depression in NAFLD group and this difference was verified by chi square test (Table 3). Furthermore, patients with NAFLD had a higher risk of havin depression: RR = 1.65, 95% CI 1.16–2.36, and $p = 0.006$.

TABLE 3: Depression grades in observed and control groups.

Group	Hamilton severity*				Total
	No depression	Mild	Moderate	Severe	
NAFLD+	7	17	14	2	40
NAFLD-	18	15	3	0	36
Total	25	32	17	2	76

* Chi square = 13.91, $df = 3$, and $p < 0.0001$.

Although persons with increased body mass index (BMI), hypertension, and metabolic syndrome dominated in the NAFLD positive group, neither BMI, hypertension, nor metabolic syndrome had influence on the cognitive status. HDL/LDL ratio was not significantly related to lower MoCA index, although the risk for atherosclerosis was expressed among examinees from NAFLD positive group. Neither transaminase level nor the ratio influenced significantly the cognitive deficit.

No gender based differences were evaluated in this study.

4. Discussion

This study represents authors' effort to evaluate the cognitive status of newly recruited NAFLD patients and to correlate it to the total brain volumes, volumes of gray and white matter, and volumes of the lateral ventricles. The major finding of our study is the assessment of higher number of persons with lower cognitive abilities in NAFLD patients. Higher risk for NAFLD patients to suffer from the cognitive impairment and independent influence of NAFLD on lower cognitive scores have also been evaluated. Previous investigations also correlated the cognitive deficit with white matter changes of vascular origin [8]. Some investigations outlined the increased connection between white matter changes in menopausal and postmenopausal females [14], but, as we had not obtained statistical correlation between age and gender with any kind of volumes of interest, we did not want to involve hormonal status in our investigation. Depleted cognitive performance has been noted in NAFLD patients: increased activity of alanine (ALT) and aspartate aminotransferase (AST) correlated with poorer performance on serial digit learning test and increased activity of ALT correlated with serial digit replacement test [15]. Serial digit learning test (SDLT) is a golden standard for functional magnetic resonance memory testing and consists of three independent series of nine (sometimes eight) single digits, in which examinee must repeat correctly after maximum 12 attempts, in five to ten minutes. The other test, serial digit replacement test (SDRT), is designed for attention testing and is based on the replacement of single digit by a prescheduled sign. Obviously, NAFLD patients have a problem with memory, also found in our study, but our patients manifested less problems in postponed memory test than in attention evaluation; moreover, we found no statistically relevant correlation between transaminases activity and cognition scores. On the other side, Petta and his

coworkers (2016) [8] found no difference in cognitive state in NAFLD positive patients, with and without white matter lesions. For the mentioned evaluation, they used mini mental state questionnaire, applied mostly to estimate the presence of dementia. This is not a sensitive type of questionnaire and its application is not advisable for subtle cognitive changes, as manifested in NAFLD. Reduced gray and white matter volumes, obtained in our study, combined with the increased volumes of the lateral ventricles, may be used as an explanation why total brain volume did not differ between the examinees and controls, because controls had increased volumes of gray and white matter but lower volumes of the lateral ventricles. According to VanWagner et al. (2017) [16], total brain tissue volume did not differ in groups with and without NAFLD, while gray matter perfusion was lower in NAFLD patients.

Previous reports have outlined that higher levels of adiposity including BMI, waist circumference, subcutaneous adipose tissue, and volume of adipose tissue have all been associated with lower total brain volume [17, 18]. BMI, in particular, has been associated with regional brain gray matter volume decreases, although the location and magnitude of these decreases have been inconsistent [19–22]. Some investigations reported lower gray matter volume only in those with obesity ($BMI \geq 30 \text{ kg/m}^2$) [18], although our investigation cannot confirm those findings. According to our results, BMI does not correlate with total brain, gray, or white matter volumes, nor with the depression grade.

Youssef et al. (2013) [3] published in their article that depression levels were associated with portal fibrosis grades ($p = 0.038$) and tended to be associated with hepatocyte ballooning grades ($p = 0.085$). In our study, 75% of NAFLD + examinees had depression versus 69% in cited publication [3]. Subclinical form of depression (e.g., mild + moderate) appeared in 28 out of 40 patients, while clinical form of depression (severe) was assessed in 2 patients. Our estimated risk for depression of 1.65 is related to overall NAFLD group, without difference for males and females. Gender differences in depression prevalence in NAFLD were outlined by Surdea-Blaga and Dumitraşcu (2011) [5], who stated that female NAFLD patients have higher relative risk for depression: 3.2 (95% CI = 1.6–6.7).

The previous investigation blamed insulin resistance for the predicted cognitive deficit in NAFLD + patients, indicating the prominent role of insulin resistance in Alzheimer's disease [23, 24]. Other studies, however, blamed the inflammatory process for cognitive impairment, relating it to the increased concentration of cytokines and adipokines, eventually associated with dysfunctional endothelium and oxidative stress, stating that it could be in the root of cognitive dysfunction in NAFLD patients [25–29]. Thickening of carotid arteries tunica intima, also reported in NAFLD patients and in patients with cognitive deficit as well, is the probable aftermath of the aforementioned process, which, as a definitive result, gives the volume reduction of gray and white matter and, consequently, cognitive impairment [30, 31]. Inflammatory mechanism should not be neglected as one of the causes of cognitive loss, but, according to our

laboratory results, values of this inflammation marker were in the accepted range (0–10 mg/l) in both groups, without statistical difference (3.96 vs. 3.52 mg/l, examinees versus controls).

However, our study has some limitations:

- (1) This is a referral, not cohort study, restricted only to the patients referred to our department and outpatient clinics.
- (2) It is limited to newly diagnosed, therapy naive patients.
- (3) Only noninvasive tests were used for the NAFLD estimation.
- (4) The diagnosis is dependable on ultrasonographer skills and experience.

5. Conclusion

According to our results, cognitive deficit, assessed by Montreal Cognitive Assessment test, is more pronounced in persons with NAFLD. NAFLD significantly influenced the cognitive deficit and patients suffering from NAFLD had about four times higher risk to have a cognitive impairment. Depleted MoCA score correlated with the white and gray matter volume reduction. NAFLD patients are at higher risk to suffer from depression, which is, most probably, related to the revealed volume reductions as well. Nonetheless, deficit is not in a verified correlation with BMI, found to be higher in the examined group, nor with the activities of liver transaminases, although the higher concentration of AST was revealed in NAFLD patients. Future investigations should be conducted to elucidate still insufficiently known exact mechanism of cognitive deficit pathogenesis, and the therapy of NAFLD is advisable to act in such a way as to prevent it.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Research Article

Diagnostic Accuracy of Platelet Count and Platelet Indices in Noninvasive Assessment of Fibrosis in Nonalcoholic Fatty Liver Disease Patients

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Objective. Keeping in mind the rising prevalence of nonalcoholic fatty liver disease (NAFLD) and the need to establish noninvasive tests for its detection, the aim of our study was to investigate whether platelet count (PC), mean platelet volume (MPV), and platelet distribution width (PDW) can predict the presence of liver fibrosis in this group of patients. **Methods.** In 98 patients with NAFLD and 60 healthy volunteers, complete blood counts with automated differential counts were performed and values of PC, PDW, MPV, and PCT were analyzed. **Results.** Patients with NAFLD had lower PC and higher MPV, PCT, and PDW compared to the controls ($P < 0.05$). When NAFLD group was stratified according to severity of liver fibrosis, there was a statistically significant difference in the average values of PDW and PC between the groups ($P < 0.05$). **Conclusion.** Patients with NAFLD have significantly higher values of PCT, PDW, and MPV when compared to the healthy controls. Further studies are needed to establish their potential use for prediction of the degree of liver steatosis and fibrosis in NAFLD patients.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide, affecting 20–30% of population in Western countries [1]. NAFLD is a spectrum from simple steatosis with favorable prognosis to nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and its complications [2]. The hallmark of NAFLD is intrahepatic deposition of triglycerides and the leading factors in this process are insulin resistance and energy misbalance. NAFLD is considered as hepatic manifestation of the metabolic syndrome [3]. Although a liver biopsy is the gold standard for establishing the diagnosis of NAFLD, we must be aware of its invasiveness, patients' discomfort, risk of severe complications, and its high cost. Bearing in

mind the rising prevalence of NAFLD and the fact that it has become one of the most common indications for liver transplantation, there is a need to establish noninvasive diagnostic markers for early detection and monitoring of the disease progression [4–6]. Several noninvasive scores for predicting liver fibrosis have already been described: the aspartate aminotransferase to alanine aminotransferase ratio (AAR), the aspartate aminotransferase to platelet ratio index (APRI), fibrosis index (FI), fibrosis-cirrhosis index (FCI), and FIB-4 index (based on age, aspartate and alanine aminotransferase, and platelet counts). Despite the suggestion that these scores correlate with degree of liver fibrosis, there is no sufficient data supporting their everyday use, yet.

Platelets, along their well-known role in hemostasis, are active participants in the process of liver inflammation. They

promote leukocyte recruitment through hepatic sinusoids and activate effector cells [7]. Additionally, there are studies suggesting that function and morphology of platelets are altered in patients with diabetes mellitus and metabolic syndrome [8]. MPV, PDW, and PCT (platelet indices) are the indicators of platelet function and activation. It has been suggested that values of platelet indices closely correlate with the presence of insulin resistance and its severity and complications [9, 10].

The aim of our cross-sectional case control study was to evaluate whether platelet count and platelet indices can accurately predict severe steatosis and liver fibrosis in patients with NAFLD patients and to compare their diagnostic accuracy with the other noninvasive scores that have been already published and validated.

2. Patients and Methods

A total of 98 patients diagnosed with NAFLD and 60 sex- and age-matched healthy volunteers without NAFLD were included in this prospective study from March to September 2016. All patients were diagnosed with NAFLD based on history, physical examination, laboratory testing, and ultrasound imaging. The diagnosis of NAFLD required the exclusion of secondary causes of liver disease and daily alcohol consumption (≥ 20 g for men and >10 g for women) [2]. The exclusion criteria were the following: age < 18 years, presence of any other chronic liver disease (CLD), hepatocellular carcinoma, severe chronic extrahepatic disease, hospital admission due to other chronic illnesses, or presence of human immunodeficiency virus infection. All patients provided a written informed consent prior to inclusion in the study.

Data necessary for the diagnosis of the metabolic syndrome [2] were collected from the patients' records. Physical examination of each patient included body weight, height, waist circumference, calculation of body mass index (BMI), and measurement of blood pressure (BP).

Blood samples were collected after 12 hours of fasting. Analyses included PC, platelet indices, liver function tests (AST, ALT, gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP)), lipid profile, and fasting blood sugar and insulin levels. Analysis of hematological parameters along with platelets and their indices was performed in whole blood anticoagulated with EDTA within 4 hours after collection, using Coulter® LH 750 Hematology Analyzer (Beckman Coulter, USA). Analysis of biochemical parameters was performed using Olympus AU2700 (Olympus Co. Ltd., Tokyo, Japan). For each patient, we calculated homeostasis model assessment-estimated insulin resistance (HOMA-IR) [11].

Sonographic evaluation (US) of hepatic steatosis was performed using five criteria: parenchymal brightness, liver to kidney contrast, deep beam attenuation, bright vessel walls, and gallbladder wall definition [12]. Grading of diffuse hepatic steatosis was used to evaluate the extent of fatty changes in the liver. Grades I to III were defined as follows:

Grade I: increased hepatic echogenicity with visible periportal and diaphragmatic echogenicity

Grade II: increased hepatic echogenicity with imperceptible periportal echogenicity, without obscuration of diaphragm

Grade III: increased hepatic echogenicity with imperceptible periportal echogenicity and obscuration of the diaphragm [13]

For the assessment of steatosis, we used hepatic steatosis index (HSI) and NAFLD liver fat score (NAFLD-LFS) and we used NAFLD fibrosis score (NFS), APRI, FIB-4 index, and BARD score (which takes into account BMI, AAR, and presence of type II diabetes mellitus) for the assessment of fibrosis. All the above scores were calculated using standard formulas on admission (Tables 1 and 2) [11, 12].

We stratified patients with NAFLD into the two groups based on the US findings. Group 1 included patients with mild and moderate steatosis, while group 2 included patients with severe steatosis and possible fibrosis. In the absence of results from a liver biopsy, we used APRI score as validated "gold noninvasive score" to stratify our patients according to the severity of steatosis and fibrosis [11, 12, 14].

A statistical analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Basic descriptive statistics included the means, standard deviations, ranges, and percentages. Normality of the distribution was examined by the Kolmogorov-Smirnov test. The differences were considered as statistically significant if the two-tailed P value was less than 0.05. The sensitivity and specificity as well as the best cut-off value for the platelet indexes were calculated using ROC curves (AUROC).

This study was approved by the Ethics Committee of our institution in keeping with the principles of the Declaration of Helsinki (2000 revision of Edinburgh).

3. Results

Clinical, laboratory, and demographic data of patients were summarized in Table 3.

Gender, age, and mean diastolic blood pressure were similar among the NAFLD patients and the control group ($P > 0.05$). NAFLD patients had significantly higher systolic blood pressure, waist circumference, and BMI compared to the controls ($P < 0.01$). Among the biochemical variables, fasting plasma glucose, insulin levels, and triglycerides were significantly higher and high-density lipoprotein was significantly lower in NAFLD group ($P < 0.01$). NAFLD group also had lower PC and higher MPV, PCT, and PDW ($P < 0.05$).

When we stratified NAFLD patients into the two groups, we found a statistically significant difference in the average values of PDW and PC between the groups ($P = 0.04$, $P = 0.03$, and $P < 0.05$) with PDW cut-off value of 16.18 for the presence of severe steatosis and possible fibrosis with sensitivity of 88.1% and specificity of 32.6% and AUROC of 0.688 (Figure 1). There were no differences between these groups with regard to MPV and PCT ($P > 0.05$).

When these groups were further stratified according to APRI score (more than or equal to 0.7), we found a statistically significant difference in values of PC, PDW, and

TABLE 1: Formulas for calculating noninvasive scores for steatosis.

Noninvasive index of steatosis	Formula
HSI	$8 * ALT/AST + BMI + 2$ if DM + 2 if female
NAFLD-LFS	$-2.89 + 1.18 * \text{metabolic syndrome (yes = 1, no = 0)} + 0.45 * \text{type 2 DM (yes = 1, no = 0)} + 0.15Io + 0.04 * AST - 0.94 * AST/ALT$

HSI: hepatic steatosis index; NAFLD-LFS: NAFLD liver fat score.

TABLE 2: Formulas for calculating noninvasive scores for fibrosis [12, 13].

Noninvasive index of fibrosis	Formula
NFS	$1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\text{)} + 1.13 \times \text{IFG/diabetes (yes = 1, no = 0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet (}\times 10^9\text{/l)} - 0.66 \times \text{albumin (g/dl)}$
APRI	$[(AST/\text{upper normal limit AST}) \times 100]/\text{platelets (}\times 10^9\text{/L)}$
FIB-4	$(\text{Age} \times \text{AST})/(\text{platelets} \times \text{sqr (ALT)})$
BARD	BMI $\geq 28 = 1p$, AST/ALT ratio (AAR) $\geq 0.8 = 2p$, DM = 1p

NFS: NAFLD fibrosis score; APRI: AST platelet ratio index; FIB-4: fibrosis-4; BARD: BMI, AST/ALT ratio, and diabetes [12, 13].

TABLE 3: Clinical, laboratory, and demographic data of NAFLD patients compared to controls.

	NAFLD	Control cases	P value
Age	51.8 ± 14.6	50.4 ± 14.1	>0.05
Gender: male/female	56/42	32/28	>0.05
BMI	29.3 ± 4.7	26.2 ± 3.6	<0.01
Waist circumference	109.4 ± 8.9	108.2 ± 6.6	<0.05
Systolic BP	130.3 ± 8.9	124 ± 6.6	<0.01
Diastolic BP	82.6 ± 6.4	81.5 ± 4.4	>0.05
Glucose	6.6 ± 2.04	5.8 ± 1.5	<0.01
Urea	6.5 ± 5.9	6.6 ± 7.5	>0.05
Creatinine	77.5 ± 15.2	73.3 ± 21.1	>0.05
Total cholesterol	5.78 ± 1.1	5.72 ± 1.4	>0.05
LDL cholesterol	3.6 ± 0.9	2.6 ± 1.1	<0.01
HDL cholesterol	1.2 ± 0.6	1.7 ± 0.4	<0.01
Triglyceride	2.4 ± 1.7	1.7 ± 0.9	<0.01
AST	33.1 ± 19.3	22.3 ± 11.2	<0.01
ALT	46.1 ± 25.8	22.1 ± 7.8	<0.01
ALP	72.3 ± 23.0	73 ± 19.2	>0.05
GGT	73.6 ± 82.9	44 ± 12.1	<0.01
WBC	7.02 ± 1.7	7.2 ± 2.6	>0.05
PC	218.4 ± 56.8	255.3 ± 77.9	<0.01
MPV	9.1 ± 1.3	7.6 ± 1.1	<0.01
PDW	16.7 ± 0.7	15.9 ± 0.7	<0.01
PCT	0.2 ± 0.1	0.1 ± 0.0	<0.01

PCT between the two groups ($P = 0.00$, $P = 0.00$, $P = 0.006$, and $P < 0.05$) (Table 4).

We found a statistically significant negative correlation between PC and APRI ($P = 0.00$; $r = -0.530$), FIB-4 ($P = 0.00$; $r = -0.480$), and NFS ($P = 0.00$; $r = -0.320$) scores, respectively. Additionally, we found statistically significant negative correlations between PDW and APRI ($P = 0.00$;

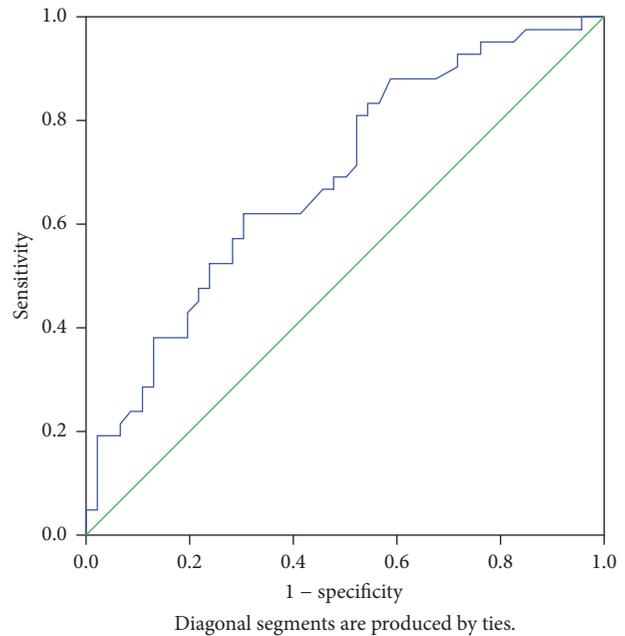


FIGURE 1: ROC curve 1. Receiver Operating Characteristic (ROC) Curve illustrates sensitivity and specificity of mean PDW values for detection of mild, moderate, and severe steatosis.

$r = -0.5629$), FIB-4 ($P = 0.00$; $r = -0.553$), and NFS ($P = 0.00$; $r = -0.346$) scores, respectively.

The results of our study suggest that there is a statistically significant negative correlation between PCT and PDW ($P = 0.06$; $r = -0.252$), as well as a significant positive correlation between PDW and MPV ($P = 0.04$; $r = 0.261$).

4. Discussion

The ability to determine the degree of the liver steatosis and fibrosis as well as to predict the progression of disease is

TABLE 4: APRI score according to platelet count and indices.

	APRI \geq 0.7	APRI $<$ 0.7	P
PC (mean \pm SD)	154.71 \pm 44.92	226.91 \pm 52.90	$<$ 0.01
MVP (mean \pm SD)	9.21 \pm 1.05	9.04 \pm 1.46	0.581
PCT (mean \pm SD)	0.14 \pm 0.03	0.22 \pm 0.16	$<$ 0.01
PDW (mean \pm SD)	16.91 \pm 0.35	16.55 \pm 0.77	$<$ 0.01

essential in the management of patients with NAFLD. A liver biopsy has been used as a gold standard for this purpose over many years. However, its invasive nature, high cost, and risk for development of severe complications (bleeding in particular) resulted in the development of noninvasive tests. These tests consist of different scores derived from various combinations of serologic markers as well as noninvasive imaging modalities [15]. Over the last decade, particularly promising imaging modality has emerged, ultrasound-based transient elastography (TE) with controlled attenuation parameter (CAP). This technique is based on mechanical excitation of the hepatic tissue with monitoring of the resulting tissue response. The fact that fibrotic tissue differs from healthy tissue in the way that it responds to excitation is used to determine level of steatosis and fibrosis. This technique is particularly useful, since it eliminates sampling errors, which was common with liver biopsy, in addition to its noninvasiveness [16, 17]. However, while it is being used more commonly, high cost of the machine is still an obstacle for use in many developing countries. Hence, there is an ongoing interest for discovery of the cheaper and easily available serologic tests for detection of liver steatosis and fibrosis and estimation of the severity grades. Multiple serologic markers reflective of liver function (AST, ALT, bilirubin, PC, GGT, ALP, and haptoglobin) have been used in combination to formulate diagnostic and prognostic scores that would be an alternative to liver biopsy. While some of them are more simple (AAR), some of them include complicated algorithms and multiple variables (FibroTest).

The current research studies suggest that platelets have a role in the process of liver fibrosis by decreasing expression of the principal fibrogenic cytokine TGF- β and by increasing expression of matrix metalloproteinases [18, 19]. Subsequently, an inverse correlation occurs between progression of liver fibrosis and platelets. Taking this into account, PC is presently included in many prognostic scores for fibrosis and cirrhosis of the liver. Some previous studies have described that lower PC is related to the more advanced fibrosis; however, only few of these studies assessed PC in NAFLD patients [20]. Unlike platelets, the platelet indices are not widely investigated as the markers of liver steatosis and fibrosis. They might prove to be very useful in the future as a part of diagnostic scores for detection of liver steatosis and fibrosis in patients with NAFLD.

Our study aimed to determine the association between PC and platelet indices with the presence of fibrosis in NAFLD patients and we found an inverse correlation between PC and liver fibrosis, similar to previously published data [18, 19].

Platelet functions can be affected by platelet size, density, other comorbidities, and age. Larger platelets have higher quantity of granules and adhesion receptors, which results in an increase in platelet activation [21]. PDW directly refers to platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology [7]. Our study suggests that NAFLD patients have higher values of PDW compared to controls. Study of Cao et al. showed that PC and PDW negatively correlate with the stage of fibrosis, which is in accordance with results that we found in the present study [22].

The results of Ozhan et al. suggest that lower PC and higher MPV are independent predictors of NAFLD [23]. Several independent studies have reported that steatosis was associated with an elevation in MPV [23–27]. A large Korean study has demonstrated a significant association between the presence of NAFLD and higher MPV values in 628 obese volunteers [24]. In our study, the NAFLD group had significantly higher values of MPV compared to the controls, which is similar to the published data.

To the best of our knowledge, there are no studies that investigated potential use of PCT for estimation of the degree of liver steatosis/fibrosis in patients with NAFLD. In the current study, we have not found any significant difference in the values of PCT between NAFLD groups 1 and 2; however, we have found significant difference in the values of PCT between NAFLD patients and the controls. This can be potentially useful as quick and simple parameter for orientation towards patients with suspected NAFLD.

5. Conclusion

In conclusion, our study demonstrates that patients with NAFLD have significant increase in the values of PCT, PDW, and MPV. We will need larger studies to investigate potential use of PC and platelet indices and their inclusion in the diagnostic algorithms for noninvasive assessment of degree of steatosis and fibrosis in NAFLD patients. Their use may be beneficial considering that they are simple, easy to measure, and cost-effective and are routinely checked in everyday practice.

Additional Points

Limitations of the Study. NAFLD diagnosis was not confirmed by liver biopsy and baseline analyses may not reflect patient's condition over prolonged period of time. Additionally, low AUROC suggest that there is a need for a larger cohort.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Research Article

Outcomes following Serial Intra-gastric Balloon Therapy for Obesity and Nonalcoholic Fatty Liver Disease in a Single Centre

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Background. The incidence of nonalcoholic fatty liver disease (NAFLD) continues to parallel the rise in obesity rates. Endobariatric devices such as the intra-gastric balloon (IGB) may provide an alternative treatment option. **Methods.** Outcomes following IGB treatment in 135 patients with obesity and NAFLD (mean baseline weight 117.9 kg; BMI 41.7 kg/m²; HOMA-IR 3.6) were retrospectively examined. Clinical, anthropometric, and biochemical changes were analysed at six months and after consecutive treatment with two and three serial IGBs. **Results.** After six months, significant changes were seen with weight and BMI (mean reductions of 11.3 kg and 4.1 kg/m², resp., $p < 0.01$ for both). Significant improvements were also seen with ALT, GGT, and HOMA-IR, with all changes corresponding with weight loss. Forty-eight patients received two IGBs, and 20 were treated with three serial IGBs. The greatest amount of total weight loss was observed after the first 6 months (mean weight lost 7.4 kg, versus 3.6 kg and 1.9 kg with two and three IGBs, resp.). **Conclusions.** IGB therapy is an effective, alternative nonsurgical means for weight loss in the management of obesity and NAFLD over the short term, with greatest outcomes observed after six months. Improvements in insulin resistance and hepatic transaminases correlated with weight change.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is now the leading cause of chronic liver disease in most affluent and several emerging economies [1]. It is expected that NAFLD will become the main indication for liver transplantation by 2020 [2].

NAFLD is invariably linked with obesity and closely associated with other complications of the metabolic syndrome (impaired glucose tolerance, hypertension, and dyslipidaemia) [3, 4]. The estimated prevalence of NAFLD largely parallels the obesity epidemic, and although it varies across different regions of the world, the unifying trend is a rapidly increasing rate being observed worldwide [2].

Obesity is primarily caused by an imbalance in energy homeostasis, with nutrient intake exceeding energy expenditure. However, this disequilibrium likely encompasses

complex interactions with several other factors, including physical activity levels, genetics, hormonal changes, maternal and perinatal nutrition, and the gut microbiota [5–7]. The prevalence of obesity, together with its associated metabolic comorbidities, is now recognised as perhaps the most important health pandemic of the 21st century [8, 9].

Although the individual natural history of NAFLD is largely uncertain, up to 30% of patients can progress to develop nonalcoholic steatohepatitis (NASH) [10], with approximately 5% of these patients at further risk of long-term progression to cirrhosis and end-stage liver disease, including hepatocellular cancer [11, 12]. Moreover, the presence of NAFLD is itself associated with a broader range of complications, including a higher risk of cardiovascular disease (CVD) and early mortality, as well as a greater risk of extrahepatic malignancies [13, 14]. The leading cause of

morbidity and death among patients with NAFLD/NASH remains adverse cardiovascular events [15].

There is currently no single, reliable treatment for NAFLD/NASH. While several potential therapeutic options are under development, lifestyle interventions remain the most effective treatment modality. Unfortunately, effective weight loss maintenance following lifestyle interventions remains elusive for most, with the majority returning to their baseline weight by 18–24-month follow-up [16, 17].

Weight loss surgeries (such as gastric bypass, sleeve gastrectomy, and gastric banding) have been shown to improve hepatosteatosis, inflammatory changes, and fibrosis in NASH patients and are currently the only treatments that have demonstrated significant, durable weight loss over the longer term [18]. Not all patients, however, are suitable or eligible for bariatric surgery, especially given the higher risk of complications with formal surgery and general anaesthesia, as well as the greater risk of impaired wound healing in obese patients. Interim or definitive endoscopic procedures such as the intragastric balloon (IGB) may therefore offer less-invasive, reversible alternatives to effect weight loss in instances where lifestyle interventions have failed and/or the patient is not a candidate for bariatric surgery.

Several prospective studies have demonstrated the efficacy of IGBs in achieving significant weight loss. Results from a meta-analysis of 15 trials (13 cohort, 2 controlled trials) studying the efficacy of IGBs in 3608 patients reported an average 34% excess weight loss (EWL) in those receiving IGB insertion for 3–6 months, associated with mean body mass index (BMI) reduction of approximately 3.2 kg/m² [19]. A few more, albeit relatively small, studies have also reported associated improvements with features of the metabolic syndrome [20], as well as liver transaminases and indexes of insulin resistance in obese patients with NAFLD over the short-medium term [21–23].

The aim of this current analysis was to report on the experience of IGB therapy as it is offered for the treatment of obesity and NAFLD. Our primary aim was to examine the efficacy of IGBs, and serial IGB therapy in particular, as an alternative or adjunctive treatment for patients with obesity and insulin resistance, focusing on weight reduction, and associated changes in metabolic and hepatic indices, as well as safety outcomes.

2. Materials and Methods

2.1. Participants. From 2005 to 2015, 135 obese patients with NAFLD received treatment with the BioEnterics Intragastric Balloon (BIB; Allergan, Goleta, CA, USA) at a single tertiary hospital (Guy's and St Thomas' Hospital, London, UK). Patients were eligible for IGB insertion if they were ≥ 18 years of age, with a BMI ≥ 27 kg/m². The majority of patients were also insulin resistant at baseline (HOMA-IR score > 2.50), and most had failed previous attempts at weight loss through lifestyle interventions or medical therapy alone. IGB therapy was contraindicated in those with a hiatus hernia > 5 cm, previous gastric surgery, significant gastric erosions or ulceration, $>$ Grade 1 oesophagitis, active coagulopathies (including anticoagulants that could not be withheld), pregnancy,

decompensated cirrhosis, contraindications to sedation for endoscopy, and an inability to provide informed consent.

The efficacy of IGB therapy in subjects with a BMI > 27 –35 kg/m², who may not qualify for formal bariatric surgery, has been demonstrated [24]. A BMI cut-off of > 27 kg/m² was therefore chosen for patient selection in this unit.

The presence of NAFLD was determined on the basis of retrospective liver biopsies confirming $> 5\%$ hepatic steatosis, formal reports of hepatic steatosis on liver ultrasound (performed within six months from the time of IGB insertion), or a raised controlled attenuation parameter (CAP) reading of > 268 dB/m on FibroScan-CAP [25].

All experiments were conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with well-informed and written consent.

2.2. IGB Insertion. Patients initially underwent gastroscopy under conscious sedation with midazolam and fentanyl. The IGB was inserted orally in deflated form into the stomach and filled under direct endoscopic vision with 500–600 mL of normal saline and 10 mL of methylene blue fluid. At approximately 6 months following insertion, each IGB was removed during another endoscopic procedure during which balloon puncture, fluid removal, and transoral retrieval of the deflated device were undertaken using specifically designed instruments. Both IGB insertion and removal were carried out as day-only procedures, with patients discharged on the same day, provided no complications were encountered. The serial (second and/or third) IGBs were placed approximately 1–2 weeks following removal of the previous balloon, using insertion and removal procedures identical to those described above. Approval for a subsequent IGB was dependent on weight loss achieved with the first balloon, patient tolerance, and preference.

2.3. Clinical, Anthropometric, and Biochemical Measurements. Clinical, anthropometric, and biochemical data were examined retrospectively for each patient where available, and differences in outcomes were compared between baseline (prior to the first IGB insertion) and at three separate time points where appropriate: T1; after the removal of the first 1st IGB, T2; after the removal of the 2nd IGB, and T3; after removal of the 3rd IGB. The primary outcome measure was weight loss, with secondary outcome measures including any significant changes in liver function, insulin resistance, and lipid profiles. Safety outcomes were also analysed.

Weight and height measurements were undertaken using standard protocols in the endoscopy suite at baseline (just prior to the IGB insertion procedure) and following IGB removal [26]. Waist circumference was also measured using standard techniques in the outpatient clinics, usually at the time of a follow-up consultation before/after IGB insertion.

Fasting blood samples were collected at baseline and at the time of IGB removal, usually following an outpatient clinic review performed within two weeks before/after the IGB insertion. The following biochemical assays were performed: full blood count, liver function tests, renal function, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, glucose, insulin,

HbA1c, vitamin D, and CRP. Serum biochemistry was performed by the diagnostic testing laboratory at St Thomas' Hospital, London, United Kingdom.

Insulin resistance was calculated using the Homeostasis Model Assessment- (HOMA-) IR score, equation: $\text{HOMA-IR} = [\text{mean fasting insulin (mIU/L)} \times \text{mean fasting glucose (mmol/L)}] \div 22.5$. A HOMA-IR score of >2.0 has been demonstrated to accurately correlate with more invasive measures of insulin resistance, as determined through euglycemic clamps and intravenous glucose tolerance tests [27].

Controlled attenuation parameter (CAP) refers to an ultrasonographic coefficient that is affected by and directly proportional to the amount of liver fat. Consequently, CAP readings are commonly measured in conjunction with transient elastography, to quantitate hepatic steatosis. CAP values range from 100 to 400 dB/m, with higher values indicating a greater degree of liver fat. CAP measurement has now also been demonstrated to provide a further useful tool in the diagnosis and staging of NAFLD, with cut-off values > 268 dB/m accurately correlating with steatosis grades > 5 –33% [25]. FibroScan-CAP measurements (performed at the discretion of the clinician) where available in the clinical notes were collated for review as part of outcomes before/after IGB insertion and part of the process for identifying obese patients with NAFLD who could be included into this analysis.

2.4. Statistical Analysis. Statistical analysis was performed using SPSS software (SPSS version 20.0, Chicago, IL, USA). Continuous variables were analysed using one-way ANOVA and nonparametric testing, while categorical variables were assessed via Chi-square and Fisher's exact analyses. Two-tailed p values of <0.05 were regarded as significant throughout.

3. Results

3.1. Baseline Characteristics. Follow-up data from a total number of 135 patients were available for analysis (Table 1). The majority were women (71%), of Caucasian background (66%), with a mean age of 47 ± 12 years. The mean baseline weight was 117.9 ± 22.0 kg (M: 122.7 kg; F: 115.7 kg), with baseline BMI of 41.7 ± 6.6 kg/m² (M: 39.7 kg/m²; F: 42.8 kg/m²). Waist circumference measurements were also available in a subset of patients ($n = 33$), with an average recording of 124.2 ± 13.6 cm (accepted normal values being ≤ 94 cm for men and ≤ 80 cm for women). The majority of patients were insulin resistant, with a median HOMA-IR score of 3.6 and moderately abnormal liver function tests and adverse lipid profiles also apparent at baseline (Table 1). The majority of patients also had concurrent features of the metabolic syndrome (60/134, 45%), and 29% already required medical treatments for Type 2 diabetes (Table 1).

3.2. Clinical Outcomes at 6 Months (T1). The mean time at IGB removal was 5.8 ± 1.7 months following insertion (Table 1). At the end of follow-up, significant reductions were seen in both weight and BMI for the majority of the cohort, with mean weight loss of 11.3 kg (117.9 kg to 106.6 kg, $p < 0.01$)

TABLE 1: Baseline characteristics.

Clinical parameter	Patient numbers ($n = 135$)
Age (years), mean \pm SD	47.1 (12.2)
Sex	M 39 (29%), F 96 (71%)
Ethnicity	
(i) Caucasian	89 (66%)
(ii) Black	27 (20%)
(iii) Asian (Indian)	2 (2%)
(iv) Asian (oriental)	2 (2%)
(v) Latino	6 (4%)
(vi) Mixed	3 (2%)
Diabetes	39/134 (29%)
Other MetSy features	60/134 (45%)
Taking ACE-inhibitor medication	94/133 (71%)
Fasting BSL [$n = 96$] (mmol/L), mean \pm SD	6.0 \pm 2.7
Weight [$n = 134$] (kg), mean \pm SD	117.9 \pm 22.0
BMI [$n = 96$] (kg/m ²), mean \pm SD	41.7 \pm 6.6
Waist circumference [$n = 33$] (cm), mean \pm SD	124.2 \pm 13.6
Fasting cholesterol [$n = 50$] (mmol/L), mean \pm SD	4.8 \pm 1.4
Fasting LDL [$n = 41$] (mmol/L), mean \pm SD	2.7 \pm 1.1
Fasting HDL [$n = 41$] (mmol/L), mean \pm SD	1.2 \pm 0.3
Fasting triglycerides [$n = 41$] (mmol/L), mean \pm SD	1.8 \pm 1.0
Fasting insulin [$n = 83$] (mIU/L), mean \pm SD	136.0 \pm 169.0
HOMA-IR [$n = 78$], median (range)*	3.6 (2.1–5.9)
ALT [$n = 108$] (IU/L), mean \pm SD	38.9 \pm 30.6
AST [$n = 16$] (IU/L), mean \pm SD	35.1 \pm 25.2
GGT [$n = 82$] (IU/L), mean \pm SD	62.6 \pm 74.9
Fib-4 score [$n = 12$], mean \pm SD	1.5 \pm 0.7
Period of 1st IGB placement (months), mean \pm SD	5.8 \pm 1.7
Total number of balloons	One = 135, two = 48, three = 20

*25–75% interquartile ranges expressed.

and mean BMI reduction of 4.1 kg/m² (41.7 to 37.6 kg/m², $p < 0.01$), respectively (Table 2). In those with paired waist circumference measurements ($n = 33$), recordings also improved significantly following IGB therapy, 124.2 cm to 101.1 cm (mean reduction of 23.1 cm, $p < 0.05$).

Although there was a trend in the reduction of fasting serum lipids following IGB therapy, the results did not reach statistical significance (Table 2).

3.3. Clinical Outcomes following Serial IGB Therapy (T2 and T3). In this cohort of 135 patients, 67 had received only one IGB during the study period, while 48 patients received two serial IGBs, and 20 patients received three IGBs. The greatest amount of total weight loss was observed in the

TABLE 2: Clinical outcomes at 6 months.

Clinical parameter	Baseline	6 months (after IGB removal)	Mean difference	<i>p</i> value
Weight (kg)	117.9	106.6	11.3	<0.01
BMI (kg/m ²)	41.7	37.6	4.1	<0.01
Waist circumference (cm)	124.2	101.1	23.1	0.04
Fasting BSL (mmol/L)	6.0	5.4	0.6	0.12
Fasting insulin (mIU/L)	136.0	96.5	39.5	0.12
HOMA-IR*	3.6	2.6	1.0	0.03
ALT (IU/L)*	38.9	31.0	7.9	<0.01
AST (IU/L)	35.1	32.8	2.3	0.11
GGT (IU/L)*	62.6	39.1	23.5	<0.01
Fasting cholesterol (mmol/L)	4.8	5.1	-0.3	0.08
Fasting LDL (mmol/L)	2.7	2.8	-0.1	0.09
Fasting HDL (mmol/L)	1.2	1.5	-0.3	0.39
Fasting triglycerides (mmol/L)	1.8	1.4	0.4	0.22

* A significant difference in ALT and GGT was noted at follow-up in those with an elevated HOMA-IR score (i.e., insulin resistance) at baseline.

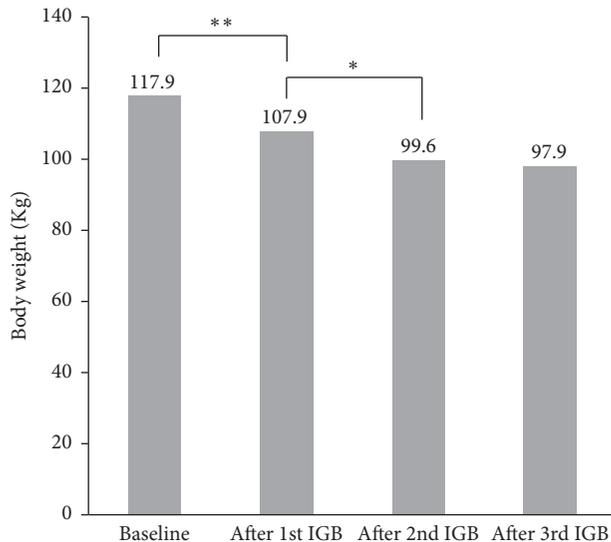


FIGURE 1: Incremental weight loss following serial IGB therapy (* $p < 0.05$; ** $p < 0.01$).

first 6 months after treatment with one IGB (T1), whereas the additional amount of weight loss achieved in those who received a 2nd (T2) and 3rd (T3) IGB were not as significant (mean weight loss of 7.4 kg versus 3.6 kg and 1.9 kg, resp.; $p = 0.05$) (Table 3). Furthermore, while the incremental weight decline following a second IGB was statistically significant, no further significant weight losses were observed in those who underwent treatment with a third IGB (Figure 1).

3.4. Changes in NAFLD Indices after IGB Treatment. After six months of IGB therapy, HOMA-IR scores were observed to significantly improve in a total of 78 patients with paired readings at baseline and final follow-up, 3.6 compared with 2.6 ($p < 0.05$). Significant improvements in serum ALT and GGT were also noted, but only when analysed in those

with an elevated HOMA-IR score at baseline. The prevalence of elevated plasma ALT and GGT concentrations decreased from 42% to 22% and from 57% to 34%, respectively, after IGB therapy at six months (Table 2). Similar findings were noted with observed changes in BMI and ALT across the groups who received serial treatment with two or three IGBs, but no further significant reductions in HOMA-IR scores were observed (Table 3).

3.5. Long-Term Follow-Up after IGB Treatment. The average period of follow-up for the total cohort was approximately 20 months. During this time, 58% ($n = 78$) of patients were lost to follow-up, and 20% ($n = 27$) were eventually referred for bariatric surgery. Of those who continued in clinical follow-up for whom data were available ($n = 37$), the mean final weight and total % of baseline weight lost at final follow-up were 108.8 kg and 6.3% for T1, 107.5 kg and 9.0% for T2, and 115.3 kg and 7.5% for T3, respectively ($p = 0.05$).

3.6. Safety Outcomes. The majority of patients tolerated IGB therapy. The most common adverse symptoms reported were nausea and vomiting (20.7%) and abdominal cramps, experienced primarily in the first few weeks after IGB insertion. During this time period, only one patient required emergency balloon removal for an unexpected gastrointestinal obstruction [28], and 14 patients (10.4%) had their IGB removed prematurely for intolerance (Table 4). There were no predominant clinical factors that predicted IGB intolerance, including gender or age.

In the patient who experienced an unexpected obstruction, the IGB was found to be significantly distended with air and fluid while still within the stomach. Balloon puncture and retrieval were performed in the standard manner, with the patient making a swift recovery after balloon extraction. Prolonged cultures of the IGB fluid did not reveal any infective (gas-forming) organisms, which raised the possibility of a defective valve potentially allowing entry of air into the device, with consequent distension.

TABLE 3: Anthropometric and ALT changes following serial IGB therapy.

Clinical parameter	After 1st IGB (T1) [n = 67]	After 2nd IGB (T2) [n = 48]	After 3rd IGB (T3) [n = 20]	p value
Final weight (kg)	107.9 ± 23.5	99.6 ± 17.1	97.9 ± 43.4	0.18
Incremental weight change*	-7.4	-3.6	-1.9	0.05 [§]
Total weight change**	-6.9	-10.6	-9.4	<0.01 [§]
Final BMI (kg/m ²)	39.0	35.5	38.2	0.15
Total change in BMI**	-2.0	-5.7	-5.5	<0.01 [§]
Final HOMA-IR	4.27	1.89	2.71	0.21
Total change in HOMA-IR**	-1.17	-1.37	-3.00	0.71
Final ALT (IU/L)	31.7	27.9	26.3	0.57
Total change in ALT**	-5.8	-4.2	21.8 [‡]	<0.01 [§]
Total follow-up (months)	17.1	18.8	26.6	
Final outcomes:				
(i) Loss to follow-up	44	25	9	
(ii) Referred for surgery	12	10	5	0.65
(iii) Still in active follow-up	11	13	6	

*Changes compared with the start weight prior to IGB insertion, and the end of the treatment period (T1/T2/T3). **Changes compared with baseline (prior to placement of the 1st IGB), and final follow-up. [‡]Data only available in 5 cases. [§]Significant changes were observed in outcomes between T1 and T2, but not between T2 and T3.

TABLE 4: Adverse outcomes.

Side effect	Number of patients
Nausea and vomiting	28 (20.7%)
Abdominal pain	8 (5.9%)
Abdominal bloating/flatulence	16 (11.9%)
Constipation or diarrhoea	6 (4.4%)
Gastroesophageal reflux	9 (6.7%)
Erosive gastritis or oesophagitis	2 (1.5%)
Deflation or displacement of IGB	1 (0.7%)
Gastrointestinal obstruction	1 (0.7%)
Premature removal	15 (10.4%)

4. Discussion

Over the next decade, obesity and its related complications will continue to instigate overwhelming health-related population and cost burdens for most economies of the world [29]. Although there is increasing recognition of this burgeoning problem, more effective treatment modalities are still desperately needed. While lifestyle interventions, focusing on dietary changes and increased exercise, remain the primary treatment recommendation, endoscopic bariatric devices such as the IGB are fast becoming a viable option for improved management through assisted weight loss [30].

To date, several studies have reported on the efficacy of IGBs in inducing significant weight loss over the short to medium term [31, 32]. Similarly, we found that IGB therapy resulted in an average weight loss of 11.3 kg and BMI reduction of 4.1 kg/m² over the initial 6 months, approximating to an average EWL (excess weight loss) of 22.5%. These findings are in keeping with figures reported by Imaz et al. [19] in their meta-analysis of several earlier studies examining the efficacy

of IGB therapy, with pooled weight and BMI losses of 14.7 kg and 5.7 kg/m², respectively.

In addition to the management of obesity and excess weight, IGB therapy appears to offer a promising adjunctive treatment modality for NAFLD/NASH, even independent of weight changes [21]. To date, only a few, relatively small studies have focused on the efficacy of IGB therapy in reducing hepatic steatosis. Forlano et al. [22] demonstrated a significant reduction in liver steatosis on serial abdominal ultrasonography in 91 “responder” patients who achieved significant weight loss after IGB treatment over 6 months. This correlated with a significant reduction in HOMA-IR scores (3.8 to 1.6, $p < 0.001$) and presumably improved insulin resistance. Similarly, Folini and colleagues [21] quantified improved hepatic steatosis on serial hepatic magnetic resonance imaging (MRI) in 18 patients who underwent IGB placement (or laparoscopic gastric banding) for 6 months, compared with no significant changes seen in 13 controls. Again, these changes correlated with improved BMI and significant reductions in weight and waist circumference.

Furthermore, in a prospective sham-controlled trial including 18 patients with obesity and NAFLD who underwent serial liver biopsies before and after 6 months of IGB placement, or sham therapy, liver histology demonstrated improved NAFLD Activity Scores (NAS) [33] in those who received IGB therapy versus no improvement in those who received sham treatment (intra-gastric normal saline solution infusion) [34]. However, no improvements in median lobular inflammation, ballooning, or fibrosis scores were seen in either group, although this may have been due to the short duration of treatment.

In our cohort, we also found improved insulin resistance through reduced HOMA-IR scores in those who achieved significant weight losses, which correlated with significant

improvements in liver function tests (i.e., serum ALT and GGT) [4]. We also observed several cases of improved hepatic NASH fibrosis with IGB therapy, as measured through serial FibroScan recordings (results not shown) in a few patients and verified with serial liver biopsy results in one case. In this particular case, originally diagnosed with decompensated cirrhosis with portal hypertension from NASH, serial IGB treatment resulted in a total weight loss of 54 kg over 19 months, with improved portal inflammation and steatosis, and fibrosis remodelling thought to indicate early cirrhosis reversal on follow-up histology. To our knowledge, this case is the first to report on regression of cirrhosis following weight loss through nonsurgical means. This case is also more significant as it demonstrates how IGB therapy can provide an alternative weight loss tool for those with advanced liver disease, and often poor levels of medical and physical fitness, for whom treatment options are often severely limited.

This study is the first to document outcomes with serial (more than two) periods of IGB therapy. In their publication examining weight loss following two IGB treatments, Genco and colleagues [35] found improved and prolonged weight loss in 50 patients who underwent a second IGB following weight loss achieved with a primary balloon, as compared with control patients who were randomised to receive only dietary therapy following their initial IGB. In the group who received two serial IGBs, the mean BMI was 30.9 versus 35.9 kg/m² ($p < 0.05$) in the control cohort at the end of approximately 13 months. In our analysis, we observed the greatest degree of weight loss following treatment with the 1st IGB (average weight loss of 7 kg posttherapy), while the amount of weight lost following the second and third IGBs was not as significant (mean weight loss of 3.6 kg and 1.9 kg, resp.), as compared with the start weights prior to each insertion period. Indeed, weight regain was actually observed in a few patients after their third IGB, which would suggest that optimal weight loss is likely achieved following two IGBs, over 12 months, while the third IGB may help to promote weight loss “maintenance” rather than further weight decline. Again, a greater magnitude of weight loss again correlated with the most significant changes in ALT, although we did not observe any concurrent improvements in HOMA-IR scores over the extended treatment period beyond the initial 6 months, likely due to the smaller degrees of weight change achieved during these later time points.

Although these results provide a promising outlook for short-medium term outcomes, the longer-term utility of IGB therapy still requires elucidation. Currently, there are few studies that report on the outcomes with IGBs beyond 12 months and none reporting on long-term outcomes with serial IGB treatments. In their report comparing weight loss outcomes on 130 patients who had retrospectively received IGB versus a matched cohort of controls who received only prospective, specialised dietary interventions, Genco et al. [36] found more significant maintenance of weight loss endpoints in the IGB group as compared with controls at both 6 months and 24 months. Similarly, a prospective analysis by Mitura and Garnysz [37] found that of 70 patients who received one IGB, at two-year follow-up, 45 patients still maintained their reduced weight, while 7 had returned to

their baseline weight, and 18 patients had experienced a “yo-yo” effect with an average weight gain of 2.7 kg.

In our cohort, over an average longer-term follow-up period of 20 months, we found that 20% of patients were unable to maintain their initial weight loss, which necessitated a referral for bariatric surgery for most. The number of IGB treatments did not appear to significantly affect the long-term outcomes in this regard, although it should be noted that almost 50% of each group had chosen to discontinue their clinical follow-up during the study period. For those in follow-up who maintained their weight loss, this seemed to plateau at 12 months (with approx. 9% of baseline weight loss) and then decline following this period. While these results might suggest that the durability of weight loss outcomes with IGB treatment can be limited in the longer term, findings to date certainly demonstrate improved weight loss maintenance as compared with current standard of care (i.e., lifestyle interventions alone) and highlight the need for more prospective, controlled trials examining the efficacy of serial IGB therapy in the long-term treatment of obesity and NAFLD. Furthermore, it reinforces the important need for supplementary, holistic measures (psychological support) to optimise the management of obesity and other metabolic diseases.

The safety of IGB therapy has been verified in several earlier studies. Although our analysis revealed a 10% premature removal rate (due primarily to intolerance), which is higher than that reported in previous publications [19], the majority of patients underwent successful, uncomplicated insertions. The main side effects of nausea, vomiting, bloating, and abdominal cramping were reported to relent by 1–3 weeks following the point of insertion.

There are several limitations to our study. Firstly, this was an uncontrolled, retrospective review; therefore, we could not directly compare our outcomes with an untreated group, and some biochemical and anthropometric indices were not available in a substantive number of patients to enable a robust analysis on such changes beyond six months of follow-up. Furthermore, the number of patients who had received two and three serial IGBs was not comparable with those who had received only one IGB, which may have diminished the statistical significance of some outcome measures. Nonetheless, our results at six and twelve months are consistent with findings described in previous reports [19, 32]. Prospective studies examining longer-term outcomes, involving two or more serial IGBs, are indeed required to further validate these important endpoints and the potential utility of IGB therapy in the management of obesity and NAFLD.

In conclusion, obesity and its related complications, including NAFLD/NASH, will continue to present a major health burden if rates continue to rise over the next decade. Weight loss remains the primary treatment recommendation for most, but modalities to affect successful and durable weight loss are limited. While medical therapies are awaited, and bariatric surgery is not a viable option for all, endoscopic bariatric devices, such as the IGB, provide an alternative or adjunctive treatment with proven efficacy in the short-medium term, with an acceptable safety profile. More studies will be required to determine their potential role as a therapeutic weight loss option in the longer term and whether

advanced metabolic liver diseases can definitely regress following effective treatment.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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