Original Article

Major Dietary Patterns in Association with the Grades of Nonalcoholic Fatty Liver Disease in Newly Diagnosed Patients Living in North of Iran

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Abstract

Background: Evidence suggests that dietary pattern is related to incidence and grades of nonalcoholic fatty liver disease (NAFLD). The aim of this study was to investigate the relationship between major dietary patterns and hepatic histologic features of newly diagnosed patients with NAFLD. **Methods:** This cross-sectional study included 260 newly diagnosed NAFLD patients. Hepatic fibrosis and steatosis were diagnosed using Fibroscan. Dietary information was obtained using a validated 168-item semiquantitative food frequency questionnaire. The association between dietary patterns and odds ratio of the grade of fibrosis and steatosis was examined by multinomial logistic regression. **Results:** The mean ± SD age participant was 46.53 ± 11.71 years. Fifty-seven percent of the participants were women. Two major dietary patterns were recognized: "Western dietary pattern" and "Mediterranean dietary pattern." After adjustment for various confounders, adherence to the two extracted dietary patterns was not associated with odds of fibrosis and steatosis ($P > 0.05$). However, P was not significant. Patients in the second quartile of the Mediterranean dietary pattern had a higher risk for being in the F1 grade compared to those in the reference group in the crude and adjusted model. **Conclusions:** The major dietary patterns of NAFLD patients living in Gilan were not related to the severity of their disease. More precise study design such as cohort or interventional studies is suggested to reveal the strength of this study findings.

Keywords: *Diet, fatty liver [Mesh], fibrosis [Mesh], dietary pattern [Mesh], Iran, nonalcoholic fatty liver disease/diet therapy [Mesh]*

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a general liver disorder.[1] The pooled overall global prevalence of NAFLD is estimated to be 25.24%[2], and a systematic review study reported that the prevalence of disease was 33.95% for the Iranian population in 2016.[3] NAFLD compasses a wide spectrum of hepatic diseases, ranging from simple steatosis to steatohepatitis, fibrosis, and finally cirrhosis and hepatocellular carcinoma.[4] This hepatic disease is a multifactorial disorder that could be impelled by the communication between environmental and genome factors.[5] Unhealthy dietary patterns and visceral fat accumulation are the key factors in the initiation and progression of steatosis to nonalcoholic steatohepatitis (NASH).[6] Various studies have examined the effect of a single or some nutrients or food groups on the risk of NAFLD.[7,8] This approach may not reflect the total diet effect due to antagonistic and synergistic interactions of dietary components. Recently, the diet has received attention as a whole concept, such as exploring major dietary patterns by several methods (such as factor analysis methods[9] or scoring the total dietary intake[10]). Findings of these methods suggested that healthy dietary patterns such as the "Mediterranean diet" and "Dietary Approaches to Stop Hypertension" diet may be related to the risk reduction of hepatic steatosis and steatohepatitis in NAFLD patients. These healthy dietary patterns mainly encompass whole grains, low-fat dairy products, olive oil, vegetables, and fruits.[9,10] Some studies investigated the relationship between dietary patterns and fatty liver disease in IRAN. A cross-sectional study on NAFLD patients reported that compliance to a healthy and western dietary pattern was correlated with the lower and higher odds of fibrosis, respectively.[5] Furthermore, another study showed that a healthy dietary pattern labeled

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as "vegetable and dairy" could reduce the risk of NAFLD but a significant relationship was not found between "fast-food" dietary pattern and the risk of diseases.[10] Iran as a developing country has been experienced a "nutritional transition" during the last decade, in which obesity and obesity-correlated non-communicable chronic diseases such as NAFLD have been rising sharply.[11] According to notable changes in Iranians' dietary patterns from relatively healthy traditional pattern characterized by high intake of dishes and cuisines prepared by vegetables and legumes to an unhealthy and western-style pattern mainly known as high intake of fats, fried and processed unhealthy fast foods,[11] the higher prevalence of NAFLD may be attributed to this eating style changes during recent years. Guilan province is located in the northern part of Iran and the dietary pattern of its residents is somewhat similar to the Mediterranean style eating pattern due to its proximity to the Caspian sea and mild and humid climate.[12] As, none of the studies have investigated the dominant dietary patterns of Guilani people and their association with the risk of NAFLD, the aim of this study is to investigate major dietary patterns of newly diagnosed patients with NAFLD living in Gilan province and their relation with the grades of disease.

Methods

Subjects

This cross-sectional study was performed on 260 (57% women and 43% men) patients newly diagnosed with fatty liver, aged 20 to 70 years, who were admitted to an exclusive private Specialized Clinic of Gastroenterology and Liver in Rasht (capital city of Guilan province in the northern part of Iran) in 2018. Patients were selected by the convenience sampling method. The aim of the study was explained to patients, and they were included in the study based on the following criteria: aged between 20 to 70 years, newly diagnosis of NAFLD. The exclusion criteria included pregnancy, breastfeeding, having infectious disease, renal diseases, inflammatory diseases, history of liver disease, taking medicine and/or supplements, having a special diet, and unwillingness to participate in the study. Individuals who had left >50% of food items blank on the food frequency questionnaire (FFQ) or those misreported energy intake outside the range between mean ± 3 standard deviation (SD).[13,14] This study was approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1397.448), and all participants gave informed consent for the study.

The sample size was calculated as follows:

The minimum number of samples size was calculated as if the minimum correlation coefficient between the two variables was equal to 0.3 (r = 0.3), considering $\alpha = 0.05$ and $\beta = 0.10$.

- $N = ((z\alpha + z\beta\sqrt{(1-r^2)})/r)^2 + 2 = 144$
- $Z\alpha = 1.96$ $Z\beta = 1.28$ $\alpha = 0.05$ $\beta = 0.10$ $r = 0.3$

The calculated sample size was 144. To predict the possible dropout and to increase the precision of this study, finally, this research was conducted on 260 people.

Study measurement

Demographic information (age, gender, marital status, education, occupation, place of residence, and family history of chronic diseases) were personally obtained from all participants using a questionnaire after explaining the goals of the study. A trained health care provider measured anthropometric data, including height (cm) (while the participants were standing against a wall no wearing shoes and with their heels and buttocks in contact with the wall) and weight (kg) (with the participants wearing light clothing and no shoes using a scale (SECA 755, Hamburg, Germany)). Body mass index (BMI) (kg/m^2) was calculated by dividing the weight in kilograms by the square of the height in meters.[15] Waist circumstance was measured to the nearest 1 mm using an inelastic plastic tape measure at the narrowest point between the lowest rib and the iliac crest, placed directly on the skin while the subject stood balanced on both feet after expiration.[16]

For each participant, samples of fasting blood were collected by trained technicians and labeled. Biochemical parameters, including liver enzymes, fasting blood sugar (FBS), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and total cholesterol, were measured.

The fatty liver was diagnosed in all subjects using transient elastography (FibroScan; Echosens, Paris, France) by a Professor of Gastroenterology and Hepatology after 8 to 10 hours of fasting. The steatosis was graded as mild steatosis (11%–34%, S1), moderate steatosis (34%–67%, S2), and marked to severe steatosis (>67%, S3).[17] Hepatic fibrosis was staged to convert Fibroscan results (measured in kPa) to the Metavir scoring system as follows: F0 (no fibrosis), F1 (mild fibrosis without septa), F2 (moderate fibrosis with few septa), F3 (severe fibrosis with numerous septa without cirrhosis), and F4 (cirrhosis or advanced scarring of the liver).[18]

Dietary assessment

Dietary data were collected using a validated 168-item semiquantitative FFQ.[19,20] Participants were asked about their frequency of intake of each food group during the previous 12 months according to a standard portion size. Afterward, the reported frequency of each food item was converted to its daily intake value and portion sizes of consumed foods were converted to grams using Iranian household scales.[19] Finally, food items were categorized into 23 predefined food groups. Food grouping was performed based on previous literature.[21,22] Energy intake per day was calculated based on USDA dietary database adapted for the Iranian food composition table.

Physical activity assessment

The physical activity of participants during last week was measured by the International physical activity questionnaire validated in the Iranian population.[23] This questionnaire has four domains (work activity, activity during transportation, activity in home, and leisure time). For each activity, metabolic equivalent thermogenesis (MET) was defined and total physical activity per week was calculated by computing MET/hour of each activity in a week.

Statistical analysis

Data were analyzed using SPSS software V.16 (SPSS Inc., USA). To compare general characteristics within the two genders, independent sample T-test and Chi-square test were used wherever applicable. Principal component analysis was applied on 23 predefined food groups [Table 1] to explore major dietary patterns. Varimax rotation was used to create the factors that explained the maximum variability of the data. Selection of the factors were based on the Scree plot and interpretably. Varimax rotation was applied as a solution to obtain an orthogonal rotation to simplify the factor structure and make it more interpretable. Factors were named based on food items highly loaded for each factor and the earlier literature. The factor score for each pattern was calculated by summing intakes of food groups weighted by factor loading[24], and each individual received a factor score for each extracted pattern. Subsequently, the total score of each pattern was categorized into quartiles. To compare general characteristics, liver's histologic features and anthropometric measures of the two genders across quartiles of identified patterns, one-way ANOVA, and Chi-square tests were used wherever applicable. Multinomial logistic regression method was used to estimate the odds ratios (OR) and 95% confidence interval (95% CI) for steatosis and fibrosis grades according to quartiles of defined dietary patterns. This relationship was adjusted by some possible cofounders in two models [model 1 adjusted for age, gender, energy intake, physical activity, BMI and waist circumferences, and model 2 further adjusted for history of disease (gallstones, hepatitis, IBD, thyroid disfunction, CVD, and diabetes), family history, smoking, supplement use, having a special diet during last year and socioeconomic status (SES)]. Scoring the SES was explained in detail previously.[13] $P < 0.05$ was considered as significant.

Table 1: Food grouping used in the dietary patterns

Food groups	Food items
Egg	Egg
Red meat	Beef, mutton, minced meat, sausage, hamburger
Refined grain	Lavash, barbary, taftoon, baguette, toast, rice, pasta, vermicelli, noodle soup, wheat flour, biscuits, crackers, corn
Snacks	Puffs and chips
Visceral meat	Heart, liver, offal, tongue and brain, and abomasum
Sweets	Types of cakes and cookies, dry sweets, wet sweets, sugar, sugar, sugar, turmeric, candy, candy, sugar halva, chocolate, honey, jam, soft drinks
French fries	French fries
Animal and hydrogenated fat	Solid vegetable oil, animal oil, animal butter, margarine
Mayonnaise	Mayonnaise
Whole grains	Barley
Poultry	Poultry
Tea and Coffee	Coffee and tea
Full-fat dairy	High-fat milk, high-fat yogurt, abstract yogurt, creamy yogurt, local cheese and liquvan, cream cheese, cream and buttermilk, ice cream
Potato	Potato
Legumes	Lentils, beans, peas, beans, soybeans, mung, beans, chickpeas
Olives	Green olives, olive oil
Nuts	Almonds, peanuts, walnuts, pistachios, hazelnuts, seeds, others
Fruits and dried fruits	Cantaloupe, gram, watermelon, melon, green tomato, apple, apricot, yellow plum, red plum, cherry, sour cherry, nectarine, peach, pear, fig, orange, tangerine, lemon, grape, kiwi, pomegranate, strawberry, banana, Sweet lemon, sour lemon, grapefruit, persimmon, warm, fresh berries, fresh pineapple, fresh figs, dates, nuts, natural and industrial juices, other
Fish	Fish, shrimp, tuna
Pickles	Pickles, pickles, salt
Vegetables	Cabbage, carrot, tomato, spinach, lettuce, cucumber, eggplant, onion, vegetables, green beans, peas, squash, mushrooms, peppers (greens and curds), turnips, cobs and corn, garlic
Low-fat dairy	Skim milk, low-fat milk, 2.5% fat milk, low-fat yogurt, 2.5%-fat yogurt, white cheese for breakfast (feta), curd, buttermilk
Oil	All types of liquid oils except olive

Results

Data were analyzed on 254 patients with NAFLD (145 (57%) women and 99 (43%) men). The mean ± SD age participant was 46.53 ± 11.71 years. For analysis, four subjects were excluded due to misreporting of energy intake and two people were excluded due to incomplete FFQ (>50% items blank). Western dietary patterns and Mediterranean dietary patterns were determined using principal component analysis (PCA). Western dietary pattern was highly loaded with egg, red and visceral meat, refined grains, snacks, sweets, French fries, animal and hydrogenated fat, and mayonnaise. The Mediterranean dietary pattern involved a high intake of olive, nuts, fruits, dried fruits, vegetables, pickles, and fish. The factor loadings matrix of extracted dietary patterns are presented in Table 2.

The main general characteristics of the participants are presented in Table 3. Higher percent of women had significantly higher dietary supplement consumption ($P < 0.001$), were illiterate or had elementary educations, were housewives ($P < 0.001$), and suffered from hypertension ($P < 0.01$) and hyperlipidemia ($P < 0.01$) compared to men. In contrast, a higher percentage of men had a smoking habit ($P < 0.001$) and had middle and higher education ($P < 0.001$), and were employed ($P < 0.001$). A higher percent of newly diagnosed patients were at the last grades (third and second) of steatosis. Inversely, a higher percentage of samples were at the 0 and first grade of fibrosis (data not shown). There was no significant difference between genders across fibrosis grades. Biochemical findings showed that levels of lipid profiles (LDL-C, HDL-C, and total cholesterol) were significantly higher in women and that liver enzymes (ALT and AST) were higher in men.

Table 2: Rotated factor loading matrix for the two identified dietary patterns

Food groups	Western dietary pattern	Mediterranean dietary pattern
Egg	0.589	
Red meat	0.578	0.342
Refined grains	0.516	
Snacks	0.459	
Visceral meat	0.459	
Sweets	0.454	0.372
French fries	0.408	
Animal and Hydrogenated fat	0.342	
Mayonnaise	0.323	
Whole grains	0.293	
Poultry	0.278	
Tea and coffee	0.228	
Full-fat dairy		
Potato		
Legumes		
Olives		0.705
Nuts		0.623
Fruits and dried fruits		0.538
Fish	-0.208	0.518
Pickles	0.309	0.463
Vegetables	0.207	0.342
Low-fat dairy		0.254
Oil		

[1]Values of <0.20 were excluded for simplicity. [2]The first factor explained 12.77% of the total variance, and the second explained 7.59% of the total variance

Hepatic histologic features, serum biochemical parameters, anthropometric measurements, and demographic characteristics of patients across quartiles of adherence to Western and Mediterranean dietary patterns are shown in Tables 4 and 5, respectively. Lower percent of housewives and retired and diabetic patients and higher percent of younger ages, employed subjects, and smokers were significantly at the highest quartile of Western pattern. Men who were at the highest quartile of this dietary pattern had significantly higher mean serum TG and Alp and women had lower mean serum Alp compared with those who were at the lowest quartile. Other variables were not significant based on gender analysis (except age, which was significantly different according to quartiles of the pattern in both genders). However, individuals at the highest quartile of the Mediterranean pattern had significantly higher educational level, were employed, and had higher levels of liver enzymes (ALT and AST), but steatosis and fibrosis grades were not different across the quartiles of this pattern. However, when analysis was conducted on genders separately, significantly higher percentage of women diagnosed with lower stages of fibrosis (0 and 1) were at the first quartile and higher percent of those with higher grades were at the last quartile. The OR of the fibrosis and steatosis grades according to quartiles of extracted major dietary patterns, defined by multinomial logistic regression, is shown in Table 6. Patients who were at the second quartile of the Mediterranean pattern had higher chance for being in the F1 grade (reference group = F0) in comparison with those in the reference group (first quartile). The relationship was strengthened after adjusting for potential confounders. (The crude model was also significant but it is not shown in the table). However, the trend was not significant ($P > 0.05$). Those who were at the second quartile of the Western pattern had a lower chance of being in the F4 grade in comparison with those in the first quartile in the first model; further adjustment for confounders yielded the result as not significant ($P > 0.05$). There was no significant relationship between dietary patterns and steatosis grades even after adjustment for possible confounders.

Discussion

The findings of the present study showed that two major dietary patterns were extracted in newly diagnosed

Table 3: General characteristics and anthropometric measurements of studied patients

	Women *n* (%)	Men *n* (%)	*P*	Total *n* (%)
Education				
Illiterate and Elementary school	52 (35.1)	9 (8.8)	0.000	61 (24.4)
Middle and High school	62 (41.9)	44 (43.1)		116 (42.4)
Higher education	34 (23.0)	49 (48.0)		83 (33.2)
Job				
Employed	24 (16.2)	84 (81.6)	0.000	108 (43.0)
Unemployed/Student	2 (1.4)	6 (5.8)		8 (3.2)
Housewife	104 (70.3)	0 (0.0)		104 (41.4)
Retired	18 (12.2)	13 (12.6)		31 (12.4)
House Owner (Yes)	117 (81.3)	84 (84.0)	0.75	201 (82.4)
Dietary supplements consumption (Yes)	76 (52.4)	23 (23.2)	0.000	99 (40/6)
Smoking (Yes)	1 (0.7)	22 (21.4)	0.000	23 (9.2)
History of NAFLD (Yes)	53 (36.1)	39 (37.9)	0.77	92 (36.8)
Diabetes (Yes)	35 (24.5)	16 (16.0)	0.11	51 (21.0)
Hypertension (Yes)	42 (29.4)	13 (13.0)	0.003	55 (22.6)
Hyperlipidemia (Yes)	72 (50.3)	32 (32.3)	0.005	104 (43.0)
	Mean±SD	**Mean±SD**	***P***	**Total**
Age (years)	49.08±10.70	42.88±12.16	0.000	46.53±11.71
Height (cm)	156.50±6.35	172.96±6.41	0.000	163.28±10.31
Weight (kg)	79.51±13.85	85.13±12.18	0.001	81.81±13.45
BMI (kg/m^2)	32.52±5.64	28.46±3.83	0.000	30.85±5.35
Waist circumference (cm)	105.51±11.36	100.19±9.27	0.000	103.36±10.87
Total energy intake (kcal/day)	2481.50±750.30	3061.65±779.63	0.000	2719.57±812.88
Western Dietary Pattern Score	−0.216±0.87	0.311±1.08	0.000	0.000±1.00
Mediterranean Dietary Pattern Score	−0.233±0.79	0.336±1.15	0.000	0.000±1.00
Total physical activity (MET.hour/week)	17.95±15.88	15.86±26.65	0.439	17.09±20.96

NAFLD: Nonalcoholic fatty liver disease; BMI: Body Mass Index; MET: Metabolic Equivalent

patients: Western and Mediterranean dietary patterns. After adjustment for possible confounders, there were no significant associations between both defined dietary patterns and steatosis and fibrosis grades in the studied population ($P > 0.05$).

The derived Western dietary pattern is characterized by increased intake of egg, red meat, refined grains, snacks, sweets, French fries, visceral meat, animal and hydrogenated fat, and mayonnaise. The Western dietary patterns were explored in numerous previous studies conducted on the Iranian population[5,19,25,26] or other countries.[27-32] Some dietary patterns named with different labels such as "unhealthy"[33,34] and "transitional"[13,35], and some named with similar labels such as high-carbohydrate dietary pattern[36] had similar components as those in the Western dietary pattern explored in our study.[13,33-36] In this study, participants at the higher quartile of the Western pattern had a lower chance for being at the higher grades of fibrosis and steatosis; however, the result was not significant even after adjustment for confounders. This result was inconsistent with previous studies. A cross-sectional study in China reported that the "animal food" dietary pattern, characterized by overconsumption of seaweed, mushroom, pork, beef, mutton, poultry, and eggs, was associated with a higher risk of NAFLD after adjustment for confounding factors.[37] Another study showed that adherence to the Western dietary pattern, which is highly loaded with refined grains, potato, red meat, white meat, eggs, and soft drinks, was associated with higher odds of hepatic fibrosis.[5] Another cross-sectional study showed that higher compliance to "fast-food" pattern, characterized by high intake of fast-food main dishes, sugar-sweetened soft drinks, and fried potatoes, correlated with higher odds for NAFLD after adjustment for age, sex, energy intake, physical activity level, smoking, education years, and presence of metabolic syndrome.[38]

The second dietary pattern that was highly loaded for olive, nuts, fruits, dried fruits, vegetables, pickles, and fish, was named "Mediterranean" because it is similar to the Mediterranean style of eating pattern that is typically characterized as low intake of meat and meat products, moderate consumption of ethanol (mostly from wine), and high intake of vegetables, fruits, nuts, legumes, fish, and olive oil, as detected in some studies by PCA method,[39,40] or by tools that specially developed for calculating the adherence to Mediterranean diet by scoring the main food components of this diet.[40-42] As an instance, in a Multi-Ethnic Study of Atherosclerosis (MESA), ten food groups, including vegetables (excluding potatoes), whole grains, nuts, legumes, fruits, high ratio of monounsaturated

Table 4: Comparison of general characteristics, hepatic histologic features, serum biochemical parameters, anthropometric measurements of patients across quartiles of western dietary pattern

	Women			Men			Total		
	Q1	Q4	*P*	Q1	Q4	*P*	Q1	Q4	*P*
Education									
Illiterate and Elementary school	18 (40.0)	11 (42.3)	0.313	0 (0.0)	2 (5.4)	0.151	18 (29.0)	13 (20.6)	0.210
Middle school and High school	15 (33.3)	9 (34.6)		4 (23.5)	18 (48.6)		19 (30.6)	27 (42.9)	
Higher education	12 (26.7)	6 (23.1)		13 (76.5)	17 (45.9)		25 (40.3)	23 (36.5)	
Job									
Employed	10 (22.2)	5 (19.2)	0.124	14 (82.4)	33 (89.2)	0.288	24 (38.7)	38 (60.3)	0.003
Unemployed and Student	0 (0.0)	1 (3.8)		0 (0.0)	3 (8.1)		0 (0.0)	4 (6.3)	
Housewife	28 (62.2)	20 (76.9)		-	-		28 (45.2)	20 (31.7)	
Retired	7 (15.6)	0 (0.0)		3 (17.6)	1 (2.7)		10 (16.1)	1 (1.6)	
House Owner (Yes)	38 (88.4)	18 (72.0)	0.684	12 (70.6)	29 (80.6)	0.292	50 (83.3)	47 (77.0)	0.749
Dietary supplements consumption (Yes)	24 (53.3)	11 (42.3)	0.692	7 (41.2)	7 (19.4)	0.159	31 (50.0)	18 (29.0)	0.116
Smoking (Yes)	0 (0.0)	0 (0.0)	0.363	0 (0.0)	15 (40.5)	0.003	0 (0.0)	15 (23.8)	0.000
History of NAFLD (Yes)	18 (40.0)	6 (24.0)	0.516	5 (29.4)	15 (40.0)	0.824	23 (37.1)	21 (33.9)	0.833
Diabetes (Yes)	15 (34.9)	1 (4.0)	0.016	4 (23.5)	4 (11.4)	0.718	19 (31.7)	5 (8.3)	0.010
Hypertension (Yes)	15 (34.1)	8 (32.0)	0.504	1 (6.3)	0 (0.0)	0.009	16 (26.7)	8 (13.3)	0.185
Hyperlipidemia (Yes)	26 (59.1)	7 (28.0)	0.064	5 (31.3)	13 (36.1)	0.862	31 (51.7)	20 (32.8)	0.177
Steatosis Grades									
0	9 (20.0)	3 (11.5)	0.221	6 (35.3)	8 (21.6)	0.335	15 (24.2)	11 (17.5)	0.120
1	6 (15.6)	2 (7.7)		2 (11.8)	2 (5.4)		9 (14.5)	4 (6.3)	
2	6 (13.3)	6 (23.1)		3 (17.6)	16 (43.2)		9 (14.5)	22 (34.9)	
4	23 (51.1)	15 (57.7)		6 (35.3)	11 (29.7)		29 (46.8)	26 (41.3)	
Fibrosis									
0	13 (35.1)	12 (48.0)	0.939	6 (46.2)	20 (60.6)	0.413	19 (38.0)	32 (55.2)	0.338
1	13 (35.1)	9 (36.0)		2 (15.4)	11 (33.3)		15 (30.0)	20 (34.5)	
2	4 (10.8)	2 (8.0)		2 (15.4)	1 (3.0)		6 (12.0)	3 (5.2)	
3	2 (5.4)	1 (4.0)		2 (15.4)	1 (3.0)		4 (8.0)	2 (3.4)	
4	5 (13.5)	1 (4.0)		1 (7.7)	0 (0.0)		6 (12.0)	1 (1.7)	
	Mean±SD	**Mean±SD**	***P***	**Mean±SD**	**Mean±SD**	***P***	**Mean±SD**	**Mean±SD**	***P***
Age (years)	53.02±40.55	42.92±9.83	0.001	45.53±12.74	36.62±9.04	0.000	50.97±10.76	39.22±9.80	0.000
BMI (kg/m^2)	33.39±5.49	31.78±5.29	0.412	28.08±3.16	28.22±3.29	0.403	31.91±5.48	29.69±4.55	0.121
WC (cm)	107.77±12.25	104.65±10.66	0.439	100.53±6.66	99.68±7.58	0.373	105.93±11.49	101.73±9.37	0.112
Total energy intake (kcal/day)	2023.83±517.71	3290.85±1036.53	0.000	2703.23±794.99	3420.12±764.99	0.001	2210.12±672.66	3366.77±881.56	0.000
Total physical activity (ME.hour/week)	20.54±16.94	13.84±11.51	0.399	13.52±19.48	17.78±32.84	0.947	18.61±17.79	16.15±26.14	0.904
LDL-C (mg/dl)	123.35±8.06	136.47±42.96	0.453	105.86±22.75	112.90±39.83	0.624	118.25±36.68	123.00±42.27	0.524
HDL-C (mg/dl)	51.47±12.21	49.67±9.34	0.777	42.71±9.97	43.65±9.68	0.956	48.92±12.09	46.23±9.87	0.424
Cholesterol (mg/dl)	204.12±48.83	215.40±52.54	0.680	175.86±30.80	195.65±51.11	0.409	195.88±45.59	204.11±51.91	0.337

Contd...

Table 4: Contd...

	Women			Men			Total
	Mean±SD	Mean±SD	*P*	Mean±SD	*P*	Mean±SD	Mean±SD	*P*
ALT (mg/dl)	33.00±26.26	26.35±13.17	0.878	49.50±33.53	62.79±53.87	0.370	37.55±28.81	47.68±45.48	0.279
AST (mg/dl)	33.71±27.40	21.53±6.90	0.533	61.38±72.38	40.13±29.35	0.096	41.34±44.77	32.41±24.50	0.302
FBS (mg/dl)	106.29±32.82	81.17±8.17	0.064	104.00±22.76	96.45±13.34	0.679	105.60±29.57	90.72±13.76	0.176
TG (mg/dl)	176.88±87.65	155.33±80.92	0.727	136.43±49.56	202.95±100.85	0.038	165.08±79.61	182.54±94.61	0.462
Alp (mg/dl)	223.57±92.88	177.12±53.33	0.044	168.13±61.54	209.46±54.60	0.046	208.28±88.01	196.05±55.78	0.479

LDL-C: Density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; FBS: Fasting Blood Sugar; TG: Triglyceride; Alp: Alkaline phosphatase. NAFLD: Nonalcoholic fatty liver disease; BMI: Body Mass Index; MET: Metabolic Eqivalent; WC: Waist circumstance. **P*<0.05 ***P*<0.01

to saturated fat, red and processed meat, whole-fat dairy products, fish, and alcohol, were studied. Participants with higher intakes than the median of the ten foods received 1 point, while those below the median received 0 points.[27] Obtaining higher score showed greater adherence to the Mediterranean diet. In a study conducted in Gilan province on 550 individuals with cardiovascular diseases, Mediterranean Diet Adherence Screener questionnaire was used for measuring the adherence score to this diet and the results showed that 53% of individuals had unsuitable adherence, 55% had moderate adherence, and only 2% had high adherence to Mediterranean diet.[43] Consumption of dried fruits and pickled vegetables is very common in Iranian food culture, and some healthy patterns showed higher factor loading for healthy patterns than unhealthy ones for these items.[33] As the alcohol consumption is not truly reported in the Iranian population, it was not included in FFQ and its consumption could not be compared with other extracted Mediterranean patterns.

In the current study, those at the higher quartile Mediterranean pattern had a higher chance for the higher grades of fibrosis and steatosis, *P* was not significant. As shown in Table 5, those who were at the highest quartile of the Mediterranean pattern had the highest level of ALT and ASP. Probability reason for this result may be due to refer of suspicious cases for more precise diagnostic tests such as fibroscan and steatosis test if the abnormal liver enzymes remains in higher level. The definite diagnosis of cases by sonography may make NAFLD patients more inclined toward a healthier diet. However, interventional studies showed the improving effect of the Mediterranean diet on NAFLD outcomes. Katsagoni *et al*.[44] reported that Mediterranean diet improved liver stiffness in NAFLD patients compared with only energy restricted diet in Greece. Another intervention study showed no significant changes in liver enzyme levels after 6-month adherence to the Mediterranean diet, but the steatosis was reduced.[45] Khalatbari and Soltani reported Mediterranean diet had inverse, but nonsignificant, association with levels of liver enzymes and had inverse significant association with hepatic steatosis.[40] One study reported that intake of seed oil, peanut oil, butter, sunflower oil, corn, oil, margarine, olive oil, extra virgin olive oil, and soy oil was associated with higher risk of NAFLD.[46] Olive and its derivatives are components of the Mediterranean diet.[47] Findings of a cross-sectional study in Spain showed that higher adherence to Mediterranean diet was significantly associated with less steatosis.[48] Some dietary patterns in previous studies named with different label such as “healthy“ was very similar to the Mediterranean dietary pattern.[49,50] In a cross-sectional study, Adriano *et al.* reported that the derived healthy dietary pattern (identified by high intake of fruits, vegetables/legumes, white meat, olive oil, margarine, and bread/toast) was inversely associated with risk of NAFLD in elderly.[49] Another cross-sectional study

Table 5: Comparison of general characteristics, hepatic histologic features, serum biochemical parameters, anthropometric measurements patient across quartiles of Mediterranean dietary pattern

	Women			Men			Total		
	Q1	Q4	*P*	Q1	Q4	*P*	Q1	Q4	*P*
Education									
Illiterate and Elementary school	23 (47.9)	7 (25.9)	0.181	3 (21.4)	3 (8.3)	0.057	26 (41.9)	10 (15.9)	0.000
Middle school and High school	18 (37.5)	12 (44.4)		9 (64.3)	13 (36.1)		27 (43.5)	25 (39.7)	
Higher education	23 (47.9)	7 (25.9)		3 (21.4)	3 (8.3)		26 (41.9)	10 (15.9)	0.000
Job									
Employed	5 (10.4)	7 (25.9)	0.517	7 (50.0)	33 (91.7)	0.009	12 (19.4)	40 (63.5)	0.001
Unemployed and Student	1 (2.1)	0 (0.0)		2 (14.3)	2 (5.6)		3 (4.8)	2 (3.2)	
Housewife	39 (81.3)	16 (59.3)		-	-		39 (62.9)	16 (25.4)	
Retired	3 (6.3)	4 (14.8)		5 (35.7)	1 (2.8)		8 (12.9)	5 (7.9)	
House Owner (Yes)	38 (82.6)	23 (85.2)	0.483	13 (92.9)	29 (82.9)	0.367	51 (85.0)	52 (83.9)	0.576
Dietary supplements consumption (Yes)	18 (38.3)	14 (51.9)	0.072	2 (15.4)	11 (31.4)	0.536	20 (33.3)	25 (40.3)	0.539
Smoking (Yes)	0 (0.0)	0 (0.0)	0.397	2 (14.3)	7 (19.4)	0.804	2 (3.3)	7 (11.1)	0.288
History of NAFLD (Yes)	16 (33.3)	7 (25.9)	0.420	4 (28.6)	11 (30.6)	0.460	20 (32.3)	18 (26.8)	0.188
Diabetes (Yes)	12 (26.1)	7 (28.0)	0.934	1 (7.1)	5 (14.7)	0.123	13 (21.7)	12 (20.3)	0.672
Hypertension (Yes)	18 (39.1)	5 (19.2)	0.291	2 (14.3)	3 (8.6)	0.673	20 (33.3)	8 (13.1)	0.057
Hyperlipidemia (Yes)	23 (50.0)	12 (46.2)	0.939	3 (21.4)	13 (38.2)	0.721	26 (43.3)	25 (41.7)	0.982
Steatosis Grades									
0	10 (20.8)	3 (11.1)	0.419	3 (21.4)	11 (30.6)	0.393	13 (21.0)	14 (22.2)	0.957
1	8 (16.7)	4 (14.8)		1 (7.1)	1 (2.8)		9 (14.5)	5 (7.9)	
2	12 (25.0)	4 (14.8)		3 (21.4)	12 (33.3)		15 (24.2)	16 (25.4)	
4	18 (37.5)	16 (59.3)		7 (50.0)	12 (33.3)		25 (40.3)	28 (44.4)	
Fibrosis									
0	23 (54.8)	8 (32.0)	0.020	6 (50.0)	16 (55.2)	0.461	29 (53.7)	24 (44.4)	0.357
1	11 (26.2)	4 (16.0)		4 (33.3)	9 (31.0)		15 (27.8)	13 (24.1)	
2	4 (9.5)	4 (16.0)		0 (0.0)	2 (6.9)		4 (7.4)	6 (11.1)	
3	1 (2.4)	4 (16.0)		1 (8.3)	2 (6.9)		2 (3.7)	6 (11.1)	
4	23 (54.8)	8 (32.0)	0.020	6 (50.0)	16 (55.2)	0.461	29 (53.7)	24 (44.4)	0.357
	Mean±SD	**Mean±SD**	***P***	**Mean±SD**	**Mean±SD**	***P***	**Mean±SD**	**Mean±SD**	***P***
Age (years)	49.55±10.12	49.63±10.81	0.851	45.43±15.58	41.36±10.62	0.139	48.61±11.58	44.90±11.39	0.271
BMI (kg/m^2)	32.32±5.59	32.21±5.27	0.911	28.28±4.94	28.57±3.88	0.395	31.41±5.68	30.13±4.84	0.335
WC (cm)	104.90±11.37	103.74±14.91	0.649	101.38±11.92	99.24±7.92	0.877	104.15±11.48	101.23±11.66	0.343
Total energy intake (kcal/day)	2265.75±920.11	2524.40±591.04	0.104	2605.37±536.77	3438.18±860.37	0.001	2342.44±856.856	3046.23±878.73	0.000
Total physical activity (MET.hour/week)	16.09±17.67	25.65±17.54	0.049	9.17±10.52	18.66±34.18	0.167	14.53±16.51	21.65±28.30	0.067
LDL-C (mg/dl)	53.93±26.88	63.04±25.67	0.143	59.75±25.80	56.10±24.63	0.151	55.22±26.52	59.31±25.12	0.828
HDL-C (mg/dl)	118.22±38.65	126.50±48.04	0.676	116.11±39.83	109.35±28.82	0.792	117.63±38.35	113.31±33.87	0.976
Cholesterol (mg/dl)	46.26±8.76	44.83±11.25	0.089	42.22±9.32	43.05±9.60	0.885	45.69±8.81	43.46±9.79	0.165
ALT (mg/dl)	189.30±42.03	211.50±61.70	0.234	185.90±57.88	184.55±33.99	0.943	188.27±46.47	190.77±42.11	0.746

Contd...

Table 5: Contd...

	Women			Men			Total		
	Mean±SD	Mean±SD	*P*	Mean±SD	Mean±SD	*P*	Mean±SD	Mean±SD	*P*
AST (mg/dl)	24.97±17.62	39.78±20.16	0.232	43.09±20.66	60.59±44.33	0.429	29.71±19.91	54.55±39.70	0.031
FBS (mg/dl)	21.26±8.74	41.89±26.73	0.088	29.36±15.71	47.95±49.30	0.314	23.38±11.36	46.19±43.58	0.008
TG (mg/dl)	97.59±21.54	89.67±17.97	0.765	104.80±20.64	97.89±19.16	0.923	100.26±21.11	95.83±18.84	0.910
Alp (mg/dl)	146.96±62.20	181.33±124.19	0.650	145.50±87.87	164.70±93.19	0.592	146.52±69.51	168.54±98.67	0.584

LDL-C: Density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; FBS: Fasting Blood Sugar; TG: Triglyceride; Alp: Alkaline phosphatase. NAFLD: Nonalcoholic fatty liver disease; BMI: Body Mass Index; MET: Metabolic Eqivalent; WC: Waist circumstance. **P*<0.05** *P*<0.01

reported that healthy dietary pattern (high intake of whole grains, legumes, vegetables, fish, and fruit) was inversely associated with risk of NAFLD.[50]

High antioxidant capacity, anti-inflammatory, and anti-fibrotic properties have been reported from the Mediterranean diet due to the high load of fruit and vegetables.[51] Monounsaturated fatty acids (MUFA), mostly provided from olive oil intake, is the main component of the Mediterranean diet. In animal studies, high intake of MUFA from olive oil repaired lipid profile, declined cytokine expression, liver enzymes, and hepatic TGs, thus mitigating hepatic steatosis and fibrosis.[52-54]

In this study, the frequency of younger ages, smokers, and employed subjects were significantly higher at the last quartile of the Western pattern; also, participants at the highest quartile of the Mediterranean pattern had higher educational levels and were employed in comparison to those in the lowest quartile. Yang *et al*.[37] reported that participants in the lowest quartile of the "Animal food" pattern, which is similar to the extracted Western dietary pattern, were more likely to be smokers and of lower age. "Animal food" pattern is defined by high intakes of kelp/seaweed and mushroom, pork, beef, mutton, poultry, cooked meat, eggs, fish and shrimp, beans, and grease. On the contrary, a study on the population of the Balearic Islands reported no association between adherence to the Mediterranean dietary pattern and SES and educational level.[55]

The strength of this study was performing the research on newly diagnosed NAFLD patients and adjustment of various confounders such as energy intake, anthropometric status, lifestyle SES, and history of the disease. This study has some limitations that should be addressed in future studies. First, the cross-sectional design of the study made it difficult to judge the causal association between diet and diseases. Second, the PCA method commonly consists of several arbitrary decisions, including choosing the number of factors, type of rotation, and naming the factors that may be accompanied by the arbitrary decision of researchers.[56]

Conclusions

Findings suggested that adherence to neither Mediterranean nor Western dietary patterns was associated with the severity of fibrosis and steatosis in newly diagnosed NAFLD patients living in northern Iran. It is suggested that this research be repeated by a longitudinal or prospective cohort design to assess precious association considering the effect of time.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The

Table 6: Multinomial logistic regression for investigating the Odds ratio of Fibrosis and Steatosis grades according to quartiles of extracted major dietary patterns of the studied patients

Dietary patterns	Fibrosis Grades[1]				Steatosis Grades[1]		
	1	2	3	4	1	2	3
Mediterranean dietary pattern[2]							
Model I[4]							
Q2	3.25 (1.25-8.20)*	3.27 (0.69-15.37)	4.60 (0.72-30.04)	2.48 (0.40-15.14)	0.92 (0.18-4.65)	0.59 (0.13-2.73)	1.18 (0.27-5.10)
Q3	1.06 (0.41-2.72)	2.51 (0.55-11.39)	1.4 (0.16-12.13)	0.50 (0.05-6.56)	0.98 (0.17-5.47)	0.84 (0.17-4.10)	1.05 (0.22-4.97)
Q4	1.13 (0.40-3.17)	3.70 (0.72-18.87)	5.60 (0.81-39.90)	5.80 (0.93-36.19)	0.41 (0.06-2.76)	0.81 (0.16-3.98)	1.50 (0.32-7.00)
P	0.62	0.48	0.50	0.06	0.65	1.11	0.91
Model II[5]							
Q2	4.53 (1.55-12.63)**	1.63 (0.24-11.07)	8.53 (0.81-89.00)	0.64 (0.03-10.64)	0.99 (0.18-4.65)	0.71 (0.11-4.53)	1.14 (0.19-5.10)
Q3	1.34 (0.45-4.00)	1.84 (0.28-11.74)	0.8 (0.04-15.77)	0.71 (0.03-16.80)	1.45 (0.17-5.47)	1.99 (0.23-16.78)	1.46 (0.18-4.97)
Q4	1.57 (0.48-5.16)	1.64 (0.20-13.28)	5.49 (0.41-30.37)	6.86 (0.43-36.19)	0.56 (0.06-2.76)	2.13 (0.29-15.30)	2.37 (0.35-7.00)
P	0.60	0.30	0.77	0.18	0.63	1.31	1.10
Western dietary pattern[2]							
Model I[4]							
Q2	0.89 (0.32-3.02)	0.49 (0.11-2.17)	1.27 (0.23-6.86)	0.24 (0.37-1.55)	0.94 (0.15-4.94)	0.70 (0.13-3.69)	1.05 (0.23-6.62)
Q3	1.07 (0.37-3.06)	1.90 (0.46-7.90)	2.13 (0.32-13.81)	1.19 (0.21-6.55)	0.71 (0.16-13.15)	1.43 (0.27-7.38)	0.79 (0.16-11.65)
Q4	0.99 (0.40-3.17)	0.56 (0.08-3.64)	1.14 (0.10-12.19)	0.31 (0.02-4.67)	0.46 (0.06-6.33)	1.89 (0.30-11.69)	1.43 (0.24-15.87)
P	0.51	0.46	0.39	0.32	0.74	1.35	0.80
Model II[5]							
Q2	0.83 (0.28-2.39)	0.83 (0.13-5.04)	2.31 (0.26-20.59)	0.05 (0.003-0.96)*	1.04 (0.13-8.24)	0.75 (0.09-5.78)	1.29 (0.19-8.72)
Q3	1.06 (0.31-3.53)	3.61 (0.56-23.02)	3.67 (0.33-40.00)	3.92 (0.45-33.56)	0.80 (0.10-4.40)	1.21 (0.17-8.72)	0.86 (0.12-5.87)
Q4	0.69 (0.18-2.61)	1.15 (0.10-12.50)	1.26 (0.06-24.70)	0.40 (0.01-10.68)	0.33 (0.02-4.59)	0.94 (0.09-9.85)	0.81 (0.08-8.03)
P	0.60	0.57	0.31	0.30	0.71	0.93	0.78

NS: Not Significant. [1]Grade 0 was considered as the reference group. [2]First Quartile was considered as the reference group. [4]Adjusted for age, gender, energy intake, physical activity, BMI, and waist circumferences. [5]Adjusted for History of disease (gallstones, hepatitis, IBD, thyroid dysfunction, CVD, and diabetes), family history, smoking, supplement use, having a special diet during last year, and socioeconomic status. *P<0.05, **P<0.01

patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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