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Cross-Sectional Study to Determine Risk Factors for Development of Non-Alcoholic Fatty Liver Disease in Type 2 Diabetic Patients

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Abstract

We designed the cross-sectional study to evaluate risk factors for development of nonalcoholic fatty liver disease in type 2 diabetic patients. In this prospective study, three hundred fifty-eight newly diagnosed type 2 diabetic male patients were evaluated. The patients were divided into two groups including non-alcoholic fatty liver (NAFLD) and nonfatty liver diseases (non-NAFLD) were evaluated for anthropometric and clinical features, laboratory findings, and nutritional characteristics. The study included 358 patients with type 2 diabetes mellitus. Two hundred sixteen (60.3%) patients had fatty liver disease whereas 142 (39.7%) patients had no fatty liver on ultrasonographic examination. The Alanin aminotransteraz GGT and LDL-C levels were found to be higher and HDL-C level was lower in NAFLD group, and the difference between (ALT) results were found to be statistically significant(p<0.05). The variables of body composition; waist circumference and waist-to-hip ratio in both group were found statistically different (p<0.05). The results of the current study showed that a higher intake of saturated, polyunsaturated fat and red meat protein were significantly associated with an increased risk for NAFLD, while a higher intake of white meat protein," milk, yogurt, cheese and egg " protein, vegetable protein and fiber tended to reduce the risk for NAFLD. The higher intake of saturated fat and lower intake of white meat, "milk, yogurt, cheese and egg "protein, vegetable protein and fiber are associated with an increased risk of NAFLD.

Key Words: Type 2 Diabetes Mellitus, NAFLD, BMI, Nutrients.



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Introduction

Fatty liver or hepatic steatosis is characterized by diffuse accumulation of fat in hepatocytes. Fatty liver occurring in individuals without a history of significant alcohol intake is called as non-alcoholic fatty liver disease (NAFLD). NAFLD is strongly associated with obesity, type 2 diabetes mellitus (DM) and hyperlipidema (1). Obesity, insulin resistance, and increased concentrations of plasma fatty acids are considered to increase the risk for fatty liver and these are also characteristics of type 2 DM. In some individuals, fatty liver can lead to steatohepatitis and may progress further to end-stage liver disease; however, many clinical symptoms of fatty liver are nonspecific or silent (2).

Liver disease is an important cause of death in type 2 diabetes. Virtually the entire spectrum of liver disease is seen in patients with type 2 diabetes. This includes abnormal liver enzymes, NAFLD, cirrhosis, hepatocellular carcinoma, and acute liver failure. Thus, patients with diabetes have a high prevalence of liver disease and patients with liver disease have a high prevalence of diabetes (3).

In general, obesity, and insulin resistance are major risk factors in the pathogenesis of NAFLD. NAFLD is associated with risk factors like obesity, central obesity, diabetes, dyslipidemia (hypertriglyceridemia, low HDL cholesterol and hypercholesterolemia), metabolic syndrome, insulin resistance, hyperinsulinemia, hypertension, elevated alanine transaminase (ALT) (4). Prospective risk factors for the development of metabolic syndrome are elevated waist circumference and triglyceride levels, elevated γ -glutamyltransferase and ALT (5).



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Diet is an important component of any weight-loss regimen. Saturated fats in the diet worsen insulin resistance, whereas dietary fiber can improve insulin resistance. Chronic high-fat diet induces insulin resistance independently of obesity. Plasma glucose and triglyceride concentrations were significantly increased in high-fat diet. Visceral obesity is associated with insulin resistance induced by high-fat diet. Dietary supplementation with polyunsaturated fat may improve insulin sensitivity. However, the effects of such a dietary modification on NAFLD are unknown. The roles of specific fiber supplements designed to decrease insulin resistance or dietary fat have not been evaluated. However, there are no controlled studies of the value of diet in the management of NAFLD (6, 7).

The aim of current cross-sectional study is to determine the risk factors for development non-alcoholic fatty liver disease in type 2 diabetic patients by using Binary Logistic Regression Analysis.

Materials and Methods

Study Design and Patient Selection

This observational cross sectional study was conducted at the internal medicine department of state hospital of Nusaybin (Mardin-Turkey) and Kirikhan (Hatay-Turkey) from September 2006 to October 2009. These are government-supported hospital's outpatient clinics, and most of the health services are provided free of charge; therefore, the socioeconomic status of the patients is typically low. A total of 358 firstly diagnosed type 2 diabetic male patients were included in the study. All of the patients were Turkish men with no alcohol and drug use. Blood samples were obtained after 12 h of overnight fasting. Fasting



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blood glucose and 2-hour glucose/post-prandial glucose levels were measured to diagnose DM by using the American Diabetes Association (ADA) criteria (8). Diagnosis of NAFLD was based on ultrasound findings suggestive of fatty liver. The patients with known chronic liver disease (hepatitis B surface antigen or Anti HCV positive), and history of alcohol or drugs which cause fatty liver were excluded from the study. After the initial evaluation, which included a general and past family history, vital signs were recorded, and all patients underwent a systemic examination. A full clinical history was obtained for every patient. Because of the differences in lifestyle and nutritional habits between the sexes, only men with Type 2 diabetes mellitus were included in this study. They had not yet been treated and were not on special diets. All patients were matched by age, BMI, physical activity, socio-economic status, and life style. Especially individuals who have a different lifestyle from the study sample were excluded from the study.

Anthropometric and Clinical Characteristics

The patients were divided into two groups (NAFLD and non-NAFLD) and were further evaluated by the measurement hemoglobin A1c (HbA1c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). In each patient, the BMI (calculated as the weight in kilograms divided by the square of the height in meters) was calculated. Waist circumference was measured midway between the lateral lower rib margin and the iliac crest. The hip circumference (HC) was measured at the levels of the major



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trochanters through the pubic symphysis to the nearest 0.1 cm. The WHR was calculated as WC/HC. Blood pressure was determined after a 5-min rest. Three seated blood pressure measurements were recorded with an ERKA sphygmomanometer (ERKA, Kallmeyer Medizintechnik Gmbh & Co.KG, Germany). The average of the last two readings was the blood pressure value used in the analysis.

Dietary Assessment

A Three-Day Food Record and a Semiquantitative Food-Frequency Questionnaire (FFQ) were used for dietary assessment. Patients completed a self-administered 116-item semi-quantitative FFQ modified for Turkish food and beverages in the current study (9). Nutrient analyses were performed by a nutrient database program (BeBiS software program) designed to evaluate the Turkish foods and commercial foods (10).

Statistical Methods

The continuous variables were presented as mean \pm SD. The normality of the variables was analyzed by the Kolmogorov–Smirnov test to use parametric test. The significance of differences in means for the two groups was calculated with a Student's t test. Logistic regression analysis was used to test possible risk factors for fatty liver patients. All variables were included in the model. Backward stepwise procedure was performed in the execution of the logistic regression analysis. Odds ratios were calculated by the logistic regression method. All statistical tests were two-tailed, with the level of significance established at p \leq 0.05. All



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analyses were conducted by using the SPSS software (version 15.0, SPSS, Inc., Chicago, IL, USA) statistical package program.

Results

The study included 358 patients of type 2 diabetes mellitus with non-alcoholic fatty liver and non-fatty liver diseases. The mean and standard deviation values for age of the NAFLD patients (n=216) and non-NAFLD patients (n=142) were respectively as follows; 49.2 ± 8.8 , 48.6 ± 9.6 . The differences between two means for age was not found statistically significant (p= 0.48). Two hundred sixteen (60.3%) patients had fatty liver whereas one hundred forty-two (39.7%) patients had no fatty liver on ultrasound examination. Comparison of means of serum biochemical markers and body composition between the groups are depicted in Table 1.

The ALT, GGT and LDL-C levels were found to be higher and HDL-C level was lower in NAFLD group, and the difference between these results were found to be statistically significant (p<0.05). The variables of body composition; waist circumference and waist-to-hip ratio in both group were found statistically different (p<0.05).

Consumption of calories for NAFLD and non-NAFLD patients (2118±1052.0 kcal/day vs. 2038±958.9 kcal/day, p=0.268) was not found significant. NAFLD patients had a significantly higher consumption of carbohydrate (238.27±112.23 vs. 280.22±133.5, p<0.001).

The results of protein, fat and fiber intake of NAFLD and non-NAFLD patients were presented by Figure 1. According to the results of Figure 1 although the total protein intake



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was not significantly difference between NAFLD and non-NAFLD patients (p=0.093), subgroups of total protein; red meat protein (RMP), white meat protein (WMP), "milk, yogurt, cheese and egg" protein (MYCEP), vegetable protein (VP) were found significantly difference between two groups of patients (p<0.001). Total fat (TF) and its sub-groups saturated fat (SF), polyunsaturated fat (PF) except monounsaturated fat (MF) (p=0.752) were found also significantly difference (p<0.001). Fiber (F) in both groups was significantly difference (p<0.001).

The Binary Logistic Regression was used to select the variables independently associated with diabetic fatty liver. The results were presented by Table 2. All variables entered the model of the logistic regression as independent risk factors of the diabetic fatty liver. Saturated fat intake, ALT, LDL-C and waist circumference were positively however vegetables protein intake is negatively correlated with fatty liver. The risk variables are presented as follows; Vegetable protein intake: Odds ratio (%95CI) =2.12 (1.46-3.08) p<0.001; Saturated fat intake: Odds ratio (%95CI)=7.13 (3.28-15.50) p<0.001; ALT: Odds ratio (%95CI)= 2.66 (1.34-5.29) p<0.001; Waist circumference: Odds ratio (%95CI)= 4.75 (1.97-11.49) p<0.001.



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Variables	Fatty liver patients (n=216)	Non-fatty liver patients (n=142)	t	р
Age (years)	49.2±8.8	48.6±9.6	0.59	0.48
ALT (U/L)	59.43±20.04	40.22 ± 19.23	9.09	< 0.001
AST (U/L)	34.92±14.19	32.22±13.21	1.83	0.100
GGT (U/L)	36.5±20.8	28.4±17.8	3.93	0.002
HbA1c	10.22±2.12	9.87±1.98	1.59	0.112
ALP (U/L)	115.1±51.1	108.9±48.1	1.16	0.255
Total cholesterol (mg/dl)	199.2±80.4	190.3±78.0	1.04	0.316
HDL-C (mg/dl)	44.8±11.2	48.6±13.9	2.72	0.016
LDL-C (mg/dl)	120.6±50.1	110.3±44.2	2.04	0.022
Triglycerides (mg/dl)	174.4±95.4	160.2±84.4	1.48	0.180
BMI (kg/m ²)	28.9±5.2	27.8±5.8	1.82	0.082
Waist circumference (cm)	94.2±6.9	92.1±7.3	2.72	0.015
Waist-to-hip ratio (cm)	0.95±.07	.92±.08	3.64	0.001
Systolic blood pressure (mmHg)	138.4±15.3	136.4±13.2	1.32	0.235
Diastolic blood pressure (mmHg)	90.2±7.1	88.9±6.9	1.72	0.091

Table 1: Main features of subjects with fatty liver patients and non-fatty liver patients

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, Gamma-Glutamyl Transpeptidase; HbA1c, Hemoglobin A1c; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; ALP, Alkaline Phosphatase



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Table 2: Logistic regression analysis and outcomes

Variables	ß	S.E.	Wald	OR(%95 CI)	р
Vegetables protein intake (g)	-0.754	0.190	15.74	2.12 (1.46-3.08)	P<0.001
Saturated fat intake (g)	1.965	0.396	24.62	7.13 (3.28-15.50)	P<0.001
ALT (U/L)	0.980	0.350	7.84	2.66 (1.34-5.29)	P<0.001
LDL-C (mg/dl)	1.230	0.234	27.62	3.42 (2.16-5.41)	P<0.001
Waist circumference (cm)	1.560	0.450	12.01	4.75 (1.97-11.49)	P<0.001



Figure 1: The results of protein, fat and fiber intake of fatty liver and non-fatty liver patients



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Discussion

Previous studies reported the association between NAFLD with risk of type 2 diabetes (2, 4, 11-14), but only a few of them determined nutritional risk factors (15-17). Little is known about the effect of calorie intake and dietary composition on NAFLD.

In the present study, 358 firstly diagnosed type 2 diabetic male patients who divided into two groups (NAFLD and non-NAFLD) were evaluated for anthropometric and clinical features, laboratory findings and nutritional characteristics.

The results of the current study showed that a higher intake of saturated, polyunsaturated fat and red meat protein (RMP) were significantly associated with an increased risk for NAFLD, while a higher intake of white meat protein (WMP), "milk, yogurt, cheese and egg" protein (MYCEP), vegetable protein (VP) and fiber tended to reduce the risk for NAFLD.

An increase of saturated fat raises the OR for NAFLD by 7.13 (3.28-15.50), while decrease of vegetables protein raises the OR for NAFLD by 2.12 (1.46-3.08). Our findings of higher intake of saturated fat and poorer intake of fiber in NAFLD patients are consistent with a paper by Musso et al (18) who reported a higher intake of saturated fat and poorer intake of fiber in NAFLD patients. However our findings also are inconsistent with the findings of the same paper claims that NAFLD patients consumed a diet richer poorer polyunsaturated fat.

Cortez-Pinto et al. (19) reported that the dietary intake of NAFLD patients was richer in fat and was poorer in carbohydrate, protein and fiber. According to the results of current study dietary intake of NAFLD patients was richer in fat and was poorer in fiber and in white meat protein, "milk, yogurt, cheese and egg" protein and vegetable protein. Our results are



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similar with the results of Cortez-Pinto et al. (19). However, protein consumption is discussed in more detail in the current study. To our knowledge, association between NAFLD and detail protein intake considered in the present study has never been investigated. Intake of carbohydrate with dietary was also found significantly poorer in NAFLD patients.

According to the results of Zelber-Sagi et al. men with NAFLD consumed a significantly larger percent of protein in their diet, but with further adjustment for age and BMI this association was not found statistically significant. However, they found a significant and independent association with meat protein intake (16). In the present study, higher intake protein of white meat, "milk, yogurt, cheese and egg" and vegetables were decrease the risk of NAFLD. Dabhi et al. reported that although there is no specific management available for NAFLD, high protein diet with high protein-calorie is also helpful (20).

The results of current study showed that intake of fiber and LDL-cholesterol were found to be significantly lower in NAFLD patients. Meta-analysis by Brown et al showed that daily intake of 2 to 10 g soluble fiber significantly lowered serum total cholesterol and LDLcholesterol concentrations (21). In addition, intake of dietary fiber has beneficial effects on risk factors for developing several chronic diseases (22). These results are compatible with the results of present study.

It was shown that NAFLD patients were overweight and obese. Abnormal cholesterol, triglycerides, and HDL-cholesterol have been found for NAFLD patients. It has been also found that NAFLD subjects had abnormal liver enzymes (23,24)

The prevalence of above abnormalities was almost similar in our study population. ALT, GGT, LDL- cholesterol in NAFLD patients of the present study were found high while



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HDL-cholesterol was low. The measure of waist circumference of NAFLD patients also was found higher than non-NAFLD patients. BMI for NAFLD and non-NAFLD patients was 28.9 and 27.8 respectively. Patients in both groups were overweight.

In conclusion, NAFLD patients have a higher intake of red meat and a tendency to a lower intake of white meat, "milk, yogurt, cheese and egg" protein, vegetable protein. The higher intake of saturated fat and lower intake of white meat, "milk, yogurt, cheese and egg" protein, vegetable protein and fiber are associated with an increased risk of NAFLD.

Conflict of interest statement: None of the listed authors has any financial or other interests that could be of conflict.

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