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Low vitamin D levels are associated with increased risk for fatty liver disease among non-obese adults

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ABSTRACT – Recent data have revealed an inverse relationship between insulin resistance, which is associated with fatty liver disease, and blood 25-hydroxy-vitamin D (25(OH)D) levels. The aim of the present study was to determine the association of vitamin D levels with the presence and stage of fatty liver disease among non-obese subjects and to determine the effect of vitamin D status on fatty liver disease development. A total of 613 non-obese (body mass index <30 kg/m^2) gastroenterology and internal medicine outpatients (472 women and 141 men) were enrolled in the study. The patients' laboratory values, including liver function tests, lipid profiles, C-reactive protein, fasting blood glucose, insulin, calcium and 25(OH)D levels were studied. Low vitamin D levels, higher triglyceride levels and higher alanine aminotransferase levels were found to be the significant determinants for non-alcoholic fatty liver disease. When the patients were evaluated as low or normal vitamin D groups, low vitamin D levels was determined to be a risk factor for fatty liver disease, with an odds ratio of –1.59 (confidence interval –1.22 to –1.97). The increased risk for fatty liver disease among patients with low vitamin D status may be suggestive of mechanisms promoting fat flow and accumulation in the liver. Molecular studies are warranted to elucidate the action of vitamin D on the liver with respect to fat metabolism.

KEY WORDS: Fatty liver disease, vitamin D

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the clinicopathological conditions characterised by morphological features observed in alcohol-related liver disease in patients without significant alcohol consumption. Most liver biopsy findings of obese and diabetic patients were similar to the pathological picture of alcoholic hepatitis and therefore were named non-alcoholic steatohepatitis (NASH) by Ludwig *et al* in 1980.[1] It has been shown that NAFLD is responsible for approximately 90% of distorted liver function tests (LFTs) in patients without any known liver diseases such as viral, alcoholic and toxic hepatitis or hereditary liver diseases.[2] Nowadays, NAFLD is suggested to be the hepatic manifestation of insulin resistance and metabolic syndrome.[3] Owing to the asymptomatic nature, the actual prevalence of disease is not known exactly, but it is clear that its prevalence is increasing day by day, mostly owing to the increase in the prevalence of obesity and type 2 diabetes.[4]

Vitamin D plays a very important role in calcium and phosphorus metabolism, and is implicated in many disorders such as autoimmune, systemic and cardiovascular diseases.[5] Recent studies have focused on the extraskeletal effects of vitamin D. In addition there are studies indicating an inverse relationship between type 2 diabetes, insulin resistance, metabolic syndrome and blood 25-hydroxy-vitamin D (25(OH)D) levels.[6,7] More interestingly, in a recent study using an oral glucose tolerance test in 1,193 subjects, the cohort was divided according to serum 25(OH)D quartiles, and an improvement in all measures of glucose metabolism and estimates of insulin resistance with increasing serum 25(OH)D quartile was found.[8]

Other than insulin resistance, inflammation and oxidative stress are the other mechanisms that are also suggested to be responsible for the development of fatty liver disease.[3] These mechanisms are also linked to vitamin D deficiency because vitamin D is suggested to have an anti-inflammatory effect among macrophages.[9] Along the same line, impaired T-lymphocyte function and increased inflammation via increased proinflammatory cytokines and oxidative stress in vitamin D deficiency have also been shown.[10,11]

In the light of these data, the aim of the present study was to determine the association of vitamin D levels with the presence and stage of fatty liver disease and insulin resistance among non-obese subjects and to determine the effect of vitamin D status on fatty liver disease development.

Methods

This study was carried out in Turgut Ozal University Hospital Ankara, Turkey, between April 2009 and April 2010. A total of 613 non-obese (body mass index [BMI] <30 kg/m^2) gastroenterology and internal medicine outpatients (483 women and 130 men) were enrolled in the study. Prior to subject recruitment, the study protocol was reviewed and approved by the university ethics committee, in accordance with the ethical principles for human investigations as outlined by the Second Declaration of Helsinki. Exclusion criteria were as follows: a significant history of alcohol

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use (>30 g daily for men, >20 g daily for female), positive results for hepatitis B surface antigen or anti-hepatitis C virus antibody, autoimmune hepatitis, Wilson's disease, haemochromatosis, any chronic liver disease, malignancies, diabetes mellitus, thyroid disease or renal disease.

Height and weight of all participants were measured and BMI was calculated by dividing the weight (kg) by the square of the height (m). Waist circumference (cm) was measured at the level of the umbilicus by a single examiner with the subject in the standing position.

All of the subjects were examined after an overnight fast and the following were studied: liver function tests (including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and bilirubin levels), lipid profiles (including total cholesterol, triglyceride [TG], high-density lipoprotein [HDL] and low-density lipoprotein [LDL] levels), C-reactive protein (CRP), fasting blood glucose (FBG), insulin, calcium and 25(OH)D levels.

Insulin resistance was measured using the homoeostatic model of the assessment of insulin resistance (HOMA-IR) and was obtained by applying the following formula:[12]

$$\text{HOMA} = \frac{\text{fasting insulin (IU/ml)} \times \text{fasting blood glucose (mmol/l)}}{22.5}$$

In addition, all patients underwent a liver ultrasonography scan, and the presence and stage of fatty liver was graded by ultrasound by an experienced radiologist who was blind to the laboratory values of the patients. Liver steatosis was scored on a scale of 0 to 3 (0 = absent; 1 = mild; 2 = moderate; 3 = severe). Steatosis was graded according to Saverymuttu *et al* on the basis of abnormally intense, high-level echoes arising from the hepatic parenchyma, liver–kidney difference in echo amplitude, echo penetration into the deep portion of the liver and clarity of liver blood vessel structure.[13]

Statistical analyses

All analyses were performed with the Statistical Package for Social Sciences (SPSS) for Windows 16.0 program. Comparisons of demographic features of groups were performed with Fisher's exact test and Pearson's chi-squared tests. Significant differences were determined between the groups using ANOVA. Multivariate linear regression was used to analyse the associations, whereas comparisons between subgroups were made using two-tailed student's *t*-test. Results were expressed as mean ± standard deviation and $p < 0.05$ was considered statistically significant.

Results

There were 275 control participants and 338 patients enrolled in the study – a total of 613 volunteers, enrolled in the study. The patients included in the study mainly consisted of women (483 subjects; 78, 7%). Among women 222 (47, 0% of all women) participants had fatty liver disease, whereas among men this number was 53 (40%). General characteristics and classification according to the stage of fatty liver disease of the patients are shown in Table 1.

There were no statistically significant differences between patients and the control group with regard to age, gender, waist circumference, AST, total cholesterol, LDL cholesterol, CRP and

Table 1. Clinical and biochemical characteristics of study population.

Property	Control (n=275)	NAFLD stage 1 (n=133)	NAFLD stage 2 (n=106)	NAFLD stage 3 (n=99)
Sex (F/M) (%)	222/53 (80)	107/26 (80)	82/24 (77)	72/27 (72)
Age (years)	50.8 ± 12.2	51.2 ± 10.5	53.1 ± 9.7	56.5 ± 8.9
BMI	26.3 ± 4.1	26.5 ± 3.4	27.2 ± 2.1	28.1 ± 2.1[c]
Waist circumference (cm)	92.2 ± 8.3	94.3 ± 9.2	95.1 ± 9.3	94.9 ± 8.2
ALT (IU/l)	19.6 ± 9.2	20.4 ± 9.7	27.6 ± 9.8	28.2 ± 10.7[c]
AST (IU/l)	18.8 ± 7.6	19.8 ± 9.2	20.3 ± 11.4	22.2 ± 11.1
GGT (IU/l)	21.4 ± 11.2	24.0 ± 10.2	26.4 ± 12.4	36.2 ± 13.2[c]
Total cholesterol (mg/dl)	198.8 ± 43.2	205.1 ± 52.1	198.2 ± 51.2	203.5 ± 56.2
HDL (mg/dl)	59.2 ± 11.1	55.4 ± 9.2	54.9 ± 8.8	48.2 ± 8.7[c]
LDL (mg/dl)	118.9 ± 32.1	121.1 ± 38.7	118.2 ± 27.2	116.1 ± 25.1
Triglyceride (mg/dl)	98.4 ± 22.1	148.2 ± 29.2[a]	133.9 ± 24.3[b]	168.3 ± 25.2[c]
HOMA-IR	1.4 ± 0.8	2.1 ± 0.5	2.4 ± 0.4[b]	2.4 ± 0.3[c]
CRP	3.3 ± 2.1	3.5 ± 1.7	3.3 ± 2.2	3.6 ± 1.9
25(OH)D (ng/ml)	26.4 ± 9.8	20.0 ± 9.2[a]	13.3 ± 6.7[b]	8.8 ± 7.4[c]
Calcium	9.4 ± 1.2	9.4 ± 0.6	9.4 ± 0.5	9.7 ± 0.4

25(OH)D = 25-hydroxy-vitamin D; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CRP = C-reactive protein; F = female; GGT = gamma glutamyl transferase; HDL = high-density lipoprotein; HOMA-IR = homoeostatic model of the assessment of insulin resistance; LDL = low-density lipoprotein; M = male; NAFLD = non-alcoholic fatty liver disease.
[a]Statistically significant difference between control group and stage 1 fatty liver disease.
[b]Statistically significant difference between control group and stage 2 fatty liver disease.
[c]Statistically significant difference between control group and stage 3 fatty liver disease.

calcium levels. On evaluation of BMI, GGT, HDL cholesterol and TG levels, there was a statistically significant difference only between the control group and patients with stage 3 fatty liver disease. On determination of insulin resistance, HOMA-IR values of patients with stages 2 and 3 fatty liver disease were statistically significantly different from those of the control group. Patients with fatty liver disease had significantly reduced serum 25(OH)D levels compared with subjects without fatty liver disease and, moreover, with an increasing stage of fatty liver disease, vitamin D levels decreased significantly.

To analyse the relationship of individual parameters including HOMA-IR, CRP, ALT, AST, GGT, BMI, triglycerides, HDL and LDL levels, logistic regression analyses were performed with fatty liver disease as the dependent variable. In this analysis, low vitamin D levels, higher triglyceride levels and higher ALT levels were found to be the significant determinants for NAFLD.

In the laboratory of our hospital, vitamin D levels lower than 10 ng/ml in winter and lower than 20 ng/ml in summer are evaluated as low. In this aspect, when the patients were evaluated as low or normal vitamin D groups, low vitamin D levels were determined to be a risk factor for fatty liver disease with an odds ratio of –1.59 (confidence interval –1.22 to –1.97).

Moreover, we evaluated the menopause status of women and determined that 62 subjects (41 controls, 21 patients) were in menopause. In subgroup analysis of patients, when the subjects in menopause were excluded, we determined a similar association between vitamin D levels and fatty liver disease status (Table 2).

Discussion

In this study, we found lower vitamin D levels among patients with fatty liver disease than in the control group and, moreover, the vitamin D levels were decreasing with advancing stages of fatty liver disease among non-obese subjects. Interestingly, we discovered an increased risk for fatty liver disease among patients with low vitamin D status. Low vitamin D status was found to be an independent risk factor for fatty liver disease. To the best of our knowledge, this is the first published study to determine the association of vitamin D levels with fatty liver disease among subjects with a BMI of <30 kg/m^2. Because vitamin D is sequestered in adipose tissue, the BMI values of subjects gain importance in the evaluation of vitamin D levels and we conducted our study among non-obese subjects to diminish this effect.

Fatty liver disease has been suggested to be one of the components of metabolic syndrome and it is particularly common in this group of patients. In a study that was carried out with 144 biopsy-proven NASH patients to determine the independent predictors of hepatic fibrosis, older age, AST/ALT ratio >1, obesity and diabetes mellitus were found to be the significant predictors of severe liver fibrosis. However, in the same study, four (10.2%) of the 39 patients with biopsy-proven fibrosis had no obesity or diabetes mellitus, indicating that obesity and diabetes mellitus is not the only mechanism in fatty liver disease.[14] Similar to our results, Targher *et al* found an inverse relationship between vitamin D levels and NAFLD in a study with 60 biopsy-proven patients.[15] Moreover, in another recent larger study with 262 patients, low 25(OH)D levels were independently associated with NAFLD.[16]

On the same line as our results, in a recent cross-sectional study performed among 6,567 healthy Korean men, Rhee *et al* determined that participants with higher serum 25(OH)D_3 levels showed a significantly reduced risk for NAFLD compared with the low 25(OH)D_3 groups, independent of obesity and metabolic syndrome.[17]

There are also recent studies that indicate an inverse relationship between 25(OH)D levels and the presence of metabolic syndrome, type 2 diabetes and insulin resistance. Belenchia *et al*[18] established that after 6 months of vitamin D_3 replacement (4,000 IU daily), participants supplemented with vitamin D_3 showed increases in serum 25(OH)D concentrations, fasting insulin, HOMA-IR and leptin:adiponectin ratio among obese adolescents. At the end of this study, the authors concluded that the correction of poor vitamin D status through dietary supplementation may be an effective addition to the standard treatment of obesity and its associated insulin resistance.[18] Similarly, in an animal study with 24 mice, vitamin D_3 supplementation has been shown to be positively correlated with a decrease in blood glucose level and serum calcium level in fasting conditions, suggesting a positive influence of vitamin D on glucose homoeostasis.[19] In another recent study by Roth *et al* in a paediatric population, hypovitaminosis D was found to be a risk factor for developing insulin resistance independent of adiposity.[20] More interestingly, the key role of vitamin D in insulin secretion, in both the insulin gene and pancreatic β cells, has been determined, and in preclinical studies, phototherapy and vitamin D supplementation have been demonstrated to ameliorate NAFLD histopathology.[21]

In another animal study, the administration of 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) prevented high-fat-diet-induced body weight gain and reduced liver weight and, moreover, 1,25$(OH)_2D_3$ attenuated hepatic steatosis in a dose-dependent manner.[22] Furthermore, in that study it was shown that 1,25$(OH)_2D_3$ downregulated mRNA expression of sterol regulatory element binding protein 1c (SREBP-1c) and its target

Table 2. Vitamin D and calcium levels after the exclusion of patients in menopause.

Property	Control (n=234)	NAFLD stage 1 (n=129)	NAFLD stage 2 (n=98)	NAFLD stage 3 (n=90)
25(OH)D (ng/ml)	26.1 ± 9.5	19.9 ± 7.4[a]	13.4 ± 7.3[b]	9.7 ± 5.9[c]
Calcium	9.4 ± 0.5	9.4 ± 0.6	9.4 ± 0.5	9.7 ± 0.3

25(OH)D = 25-hydroxy-vitamin D.
[a]Statistically significant difference between control group and stage 1 fatty liver disease.
[b]Statistically significant difference between control group and stage 2 fatty liver disease.
[c]Statistically significant difference between control group and stage 3 fatty liver disease.

genes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), which are involved in lipogenesis. Peroxisome proliferator-activated receptor α (PPARα) and its target gene carnitine palmitoyltransferase-1 (CPT-1), involved in hepatic fatty acid (FA) oxidation, were also shown to be upregulated by $1,25(OH)_2D_3$. In the light of these results, the authors suggested that the preventative effect of $1,25(OH)_2D_3$ against high-fat-diet-induced hepatic steatosis was related to the inhibition of lipogenesis and the promotion of FA oxidation in rat liver.[22]

It is well known that the intestinal absorption of vitamin D could be affected in the presence of cholestasis, as dietary vitamin D absorption depends on bile salts.[23] Moreover, the liver plays an essential role in not only synthesis of vitamin D, but also its metabolism. These mechanisms may also be responsible for reduced vitamin D levels in fatty liver disease. However, we have found an increased risk for fatty liver disease among patients with low vitamin D status, indicating that vitamin D has an essential role in fat-flow regulation of the liver.

Our study has some limitations. First, the fatty liver disease was diagnosed and staged by ultrasonography, not biopsy. However in the literature, all large studies are performed with ultrasonographic data because biopsy is not indicated and feasible in this large population group. Secondly, there may be monthly differences in vitamin D measurements, but we evaluated patients as low or normal status according to the month of measurement. One further important point that should be kept in mind regarding vitamin D levels is that low vitamin D levels may be a result of probable sedentary lifestyles, and the patient or control groups were not asked any questions about their lifestyles. However, because age is an important factor in lifestyle modifications in general and the age groups were not different between the two groups studied, this effect may be neglected.

It is not possible to discuss a cause-and-effect relationship in such a cross-sectional study; however, it is clear that NAFLD is the liver involvement of a systemic disease, and considering this common pathogenesis all patients with fatty liver disease must be evaluated systematically. Moreover, the increased risk for fatty liver disease among patients with low vitamin D status may be suggestive of mechanisms promoting fat flow and accumulation in liver. Molecular studies are warranted to elucidate the action of vitamin D on the liver with respect to fat metabolism.

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