Vitamin D3 Deficiency in Non-Alcoholic Fatty Liver Disease

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Abstract- Regarding the importance of non-alcoholic fatty liver disease (NAFLD) and the high prevalence of vitamin D3 deficiency in different societies. This study aimed to evaluate the distribution of Vit D3 deficiency in individuals with non-alcoholic fatty liver disease. In this cross-sectional study, 122 individuals with non-alcoholic fatty liver disease were selected by a simple sampling method. After collecting demographic data, serum Vit 25(OH) D3 level was measured by the ELFA method. Blood lipids level (TG, cholesterol, HDL, LDL), FBS, AST, ALT, alkaline phosphatase, total and direct bilirubin, albumin, and PT were measured by the enzymatic method. To analyze the data, descriptive and analytical methods and SPSS software version 16 were used. The study cases are comprised of 122 individuals (57.4% male). The average age of cases was 42.4±11.7 years, and the mean of serum Vit D3 level was 19.8±22 ng/dl (3-220 ng/dl). Regarding the serum 25(OH) D3 levels data showed 66.4% of cases were Vit D3 deficient (Vit D3 level< 20 ng/dl), 18% had insufficient level (Vit D3 level=20-30 ng/dl), and the remained 15.6% had sufficient level (Vit D3 level> 30 ng/dl). HDL level was higher in individuals with 25(OH) D3 sufficiency compared to those with 25(OH) D3 insufficiency and Vit D3 deficiency (P=0.019). There was no significant relationship between serum Vit D3 level and other investigated variables. The results of this study indicated that most individuals with non-alcoholic fatty liver disease had Vit D3 deficiency. Further studies are suggested.

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Keywords: Vitamin D3; Non-alcoholic fatty liver disease; Liver function tests; Lipid profile

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the causes of chronic liver disease worldwide (1). The prevalence of NAFLD estimated to vary from 10-35% in the USA to 6-35% in other parts of the world (2-16). A wide spectrum of liver damage, ranging from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma (HCC), and liver-related mortality may occur during this process (17). NAFLD is considered as liver manifestation of metabolic syndrome (MS) (18).

It has been proposed that Vit D3 deficiency may increase the severity of non-alcoholic fatty liver disease (19). This vitamin plays a significant role in many crucial physiological processes, such as insulin resistance, muscle contraction, immune function, and calcium and bone metabolism (20). The results of recent studies indicate that a low serum level of Vit D3 is meaningfully related to NAFLD activity score and may increase lipid deposition in liver cells (21-23).

The prevalence of Vit D3 deficiency in different countries is estimated to be 52-72% (19). Obese individuals are prone to Vit D3 deficiency due to a high calorie diet and low levels of minerals. Excessive accumulation of lipids in the body, depending on its location, can have a reverse effect on Vit D3 status because this vitamin is fat-soluble and is sequestered by body fat (24).

Accordingly, we aimed to investigate the level of Vit D3 in individuals suffering from non-alcoholic fatty liver disease.

Materials and Methods

This cross-sectional study was conducted on 122

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individuals with NAFLD in Afzalipour academic hospital, Kerman, Iran. These individuals were assessed by physical examination, sonography, and liver function tests.

Those cases with chronic viral hepatitis, Celiac disease, metabolic bone disorders, subjects with diabetes mellitus who used insulin and/or oral glucose-lowering drugs, autoimmune hepatitis, cholestatic liver diseases, hemochromatosis, Wilson's disease, receiving drugs may alter liver enzymes and Vit D3 were excluded from the study. All of the individuals agreed and signed to participate in the study.

Demographic information was recorded in the data collection form. The blood sample was obtained from all of the patients. Vit D3 analysis was conducted in the form of 25(OH) D3, and the method used for its quantification was ELFA in reference laboratory with Vidas equipment. The serum level of 25(OH) D3 was classified into deficient (<20 ng/dl), insufficient (20-30 ng/dl), and sufficient (>30 ng/dl) according to Malabana, Chapuy and Heaney (25-27). Determination of triglycerides (TG), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), fasting blood sugar (FBS), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), total and direct bilirubin, albumin and prothrombin time (PT) were performed by the enzymatic method with Auto analyzer Technicon RA-1000 equipment.

Sonographic description for fatty infiltration of the liver was divided into mild, moderate, and severe:

Grade 1 (mild): Slight, diffuse increase in fine echoes in hepatic parenchyma; normal visualization of the diaphragm and intrahepatic vessel borders

Grade 2 (moderate): Moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm

Grade 3 (severe): Marked increase in fine echoes with poor or non-visualization of the intrahepatic vessel borders, diaphragm, and posterior portion of the right lobe (28).

Statistical analyses were performed by the SPSS software version 16 and by using Independent t-test, *Chi*-square test, and Pearson's correlation.

This study was approved by the Ethics Committee of Kerman University of Medical Sciences in Iran (Ethical Code: IR.KMU.REC.1394. 616).

Results

Description of the study population

The studied group comprised of 122 individuals with NAFLD, men (n=70, 57.4%) and women (n=52, 42.6%). The average age of cases was 42.4±11.7 years (21-86 years). The mean height, weight, body mass index (BMI), and waist circumference (WC) were 170.1, 85.5, 29.8, and 89.4, respectively. Sixteen cases had high blood pressure, 5 cases had diabetes mellitus (DM), and metabolic syndrome was observed in cases. The measured biochemical variables are shown in table 1.

Table 1. Biochemical variables in patients with NAFLD (n=122)

Variable	Mean/SD
TG (mg/dl)	187.1±95.7
HDL (mg/dl)	43.5±11
Cholesterol (mg/dl)	195.7±44.6
LDL (mg/dl)	119.6±37.8
FBS (mg/dl)	121.6±48.8
AST (U/L)	39.4±22
ALT (U/L)	58.6±37.1
Alk P (U/L)	120.3±65.9
Total bilirubin (mg/dl)	1.9±10.2
Direct bilirubin (mg/dl)	0.1±0.1
PT (s)	11.7±1.2
Albumin (mg/dl)	4.3±0.4

Vit D3 level status in the studied subjects

The average serum 25-hydroxy Vit D3 level was 19.8±22 ng/dl (3-220 ng/dl). Prevalence of sufficiency, deficiency, and insufficiency of 25(OH) D3 in the studied group was 15.6%, 66.4%, and 18%, respectively. Regarding the gender and 25(OH) D3 levels, we found a lower mean level in men (17.5±11.2 ng/dl), compared to women (22.8±31), without a significant relationship (*P*=0.189).

The relationship between Vit D3 level, biochemical variables, and anthropometric characteristics

The level of HDL was higher in individuals with 25(OH) D3 sufficiency compared to those with 25(OH)

D3 insufficiency and Vit D3 deficiency (*P*=0.019). The other biochemical variables didn't show a significant statistical relationship. These results are shown in Table 2.

Table 2. Mean serum concentration of biochemical indicators of liver tests, lipid profiles, glucose and anthropometric characteristics in relation to the nutritional status of Vit D3

Variables	Nutritional status of Vit D3			*P*
	deficiency N=81	insufficiency N=22	sufficiency N=19	
TG (mg/dl)	197.5±10.2	174.2±88.3	157.1±64.9	0.201
HDL (mg/dl)	41.5±10.5	47.3±10.8	47.6±11.6	0.019
Cholesterol (mg/dl)	195.7±43.7	195.7±54.1	195.4±38.1	0.999
LDL (mg/dl)	118.8±38.4	116.7±67.2	126.4±33	0.679
FBS (mg/dl)	121.4±46.4	126.4±67.2	116.7±33.8	0.819
AST (U/L)	41.2±23.9	32.5±8.7	39.3±24	0.264
ALT (U/L)	58.3±37.9	51.3±24	68.4±44.6	0.338
Alk P (U/L)	120±65.7	116.3±62.8	126±73.2	0.895
Total Bilirubin (mg/dl)	1.01±1.1	6.2±2.4	1.01±0.18	0.098
Direct bilirubin (mg/dl)	0.18±0.08	0.21±0.14	0.19±0.09	0.315
PT (s)	11.6±1.2	11.9±1.3	11.7±1.1	0.738
Albumin (U/L)	4.30±0.4	4.37±0.4	4.33±0.4	0.782
Weight (kg)	86.1±15.8	82.9±14.2	86.1±13	0.670
Height (cm)	170.1±8.4	168.5±8.5	171.6±7.3	0.497
Body Mass Index (BMI) (kg^2)	30.1±4.9	29.2±4.4	29.5±4.4	0.676
Waist Circumference (WC) (cm)	89.5±15.01	86.5±12.5	92.8±11.3	0.365
Systolic pressure (mmHg)	125.3±15.03	126.5±14.8	123.6±13.4	0.820
Diastolic pressure (mmHg)	77.8±10.1	80.9±9.7	75.5±8.6	0.212

The relationship between NAFLD grades and 25(OH) D3 levels.

Overall, the results showed no significant relationship between ultrasound fatty grading and 25(OH) D3 level. However, in cases with a higher grade of sonographic fatty infiltration, the 25 (OH) D3 level was lower and vice versa. (In individuals with mild grade, the percentage of deficiency, insufficiency, and sufficiency was 62.5%, 23.8%, and 13.8%, respectively. 73% of cases with moderate grade had deficient, 8.1% had an insufficient level, and 18.9% had sufficient level. The percentage of deficiency in cases with severe grade was 80%, insufficiency was 0%, and sufficiency was 20%).

Diagnosis of NAFLD grade by sonography and its relationship with liver function tests

According to sonography (TUS) findings, 20.5% of the individuals had mild, 65.6% moderate, and 13.93% severe NAFLD. Table 3 shows the frequency of NAFLD TUS grade based on AST and ALT levels. No significant relationship was observed in ALT, AST level with different NAFLD grade.

Table 3. Frequency of NAFLD TUS grade according to AST and ALT level

Variable	NAFLD grade by sonography			*P*
	Mild N (%)	Moderate N (%)	Severe N (%)	
AST				0.337
Normal	14 (18.9)	52 (70.3)	8 (10.8)	
Abnormal	11 (22.9)	28 (58.3)	9 (18.8)	
ALT				0.534
Normal	10 (25)	26 (65)	4 (10)	
Abnormal	15 (18.3)	54 (65.9)	13 (15.9)	

Discussion

The results of this research indicated the most individuals with NAFLD had Vit D3 deficiency. Similar studies indicated the same results (21,29-30). Today, Vit D3 deficiency is an epidemic event around the world and may increase lipid deposition in liver cells through an inflammatory path that intensifies non-alcoholic fatty liver disease (21). The role of serum Vit D3 was emphasized in chronic liver diseases and NAFLD in particular (20). Because Vit D3 has anti-inflammatory and immune-modulatory properties that provide credible

mechanisms that may influence disease progression and severity of NAFLD (31). Since the low serum level of 25(OH) D3 is associated with hepatic fibrosis progression, it may increase the risk of hepatocellular carcinoma. Potentially Vit D3 can inhibit hepatic fibrosis and, as a detoxifying enzyme, may be useful for the prevention of fibrosis progression in NAFLD (29,31).

However, we didn't find a significant relationship between the severity of NAFLD and serum Vit D3 level, but those individuals who suffered from severe NAFLD comprised the most percentage of Vit D3 deficient cases. Targher et al. indicated that the increase in the fatty deposition in liver tissue decreases serum Vit D3 level compared with the control group (19). Corderio *et al*., indicated a significant difference between the severity of NAFLD and serum Vit D3 level (29). Barchetta et al. study indicated an inverse correlation between serum Vit D3 level and the grade of NAFLD. Therefore, Vit D3 may have a dose-dependent effect on fat accumulation into the hepatocytes (21). The differences between this study and other similar studies are due to various factors such as genetic, geographical environment, lifestyle, diet, and medicines.

In this study, serum Vit D3 level had a significant statistical relationship with the HDL level. It had no significant statistical relationship with other investigated variables such as TG, cholesterol, LDL, FBS, liver function tests, blood pressure, and anthropometric indicators. In similar research, serum Vit D3 level had a significant correlation with waist circumference, TG, and ALT (32). The possible mechanism by which Vit D3 can be related to the TG level is through lipoprotein lipase increased activity (32). The difference between the results of this study and other studies may be due to the small sample size of this study.

In this study, AST status was normal in 70.3% of individuals with moderate NAFLD. There was no significant difference in liver enzymes activity of individuals with various grades of non-alcoholic fatty liver disease. Cordeiro *et al*., indicated that there was no significant difference in the average of ALT and AST levels of individuals with liver steatosis and steatohepatitis (29). Usually, these enzymes are normal in 78% of individuals (33). The most reason for liver enzymes elevation is a non-alcoholic fatty liver disease (34).

In our study, there was not a significant difference in the serum level of Vit D3 regarding gender. However, more of the Vit D3 deficiencies cases were men (68.6%). Similar to this finding are the results of Cordeiro and Cabral studies, which showed a higher prevalence of Vit D3 deficiency in men (29,33).

According to our findings, Vit D3 deficiency was more prevalent in non-alcoholic fatty liver disease. In order to clarify the role of Vit D3 in NAFLD, more studies with a larger sample size based on therapeutic effects are recommended.

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