Vitamin D3 Deficiency in Non-Alcoholic Fatty Liver Disease

Mohammad Mahdi Hayatbakhsh Abbasi, Mohammad Javad Zahedi, Sodaf Darvish Moghadam, Fereshteh Arab Ghahestani, Fatemeh Karami Robati

1 Department of Internal Medicine, Gastroenterology and Hepatology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran
2 Clinical Research Unit, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran

Received: 04 Apr. 2019; Accepted: 06 Dec. 2019

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 deficiency 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.

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
Vitamin D3 deficiency in NAFLD

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).

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 |       |       |       |       |
|----------------------------|------------------------------|-------|-------|-------|-------|
|                            | deficiency N=81              | insufficiency N=22 | sufficiency N=19 | P     |
| 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±0.04 | 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)              | 4.3±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) (kg²)| 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 |

### 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.

Acknowledgments

The authors thank the staff and participants of this study for their important contributions.

References

1. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865-73.
2. Amarapurkar D, Kamani P, Patel N, Gupte P, Kumar P, Agal S, et al. prevalence of non-alcoholic fatty liver disease: population based study. Ann Hepatol 2007;6:161-3.
3. Karnikowski M, Córdova C, Oliveira RJ, Karnikowski MG, Nóbrega Ode T. Non-alcoholic fatty liver disease and metabolic syndrome in Brazilian middle-aged and older adults. Sao Paulo Med J 2007;125:333-7.
4. Das K, Das K, Mukherjee PS, Ghosh A, Ghosh S, Mridha AR, et al. Nonobese population in a developing country has a high prevalence of non-alcoholic fatty liver and significant liver disease. Hepatology 2010;51:1593-602.
5. Li H, Wang YJ, Tan K, Zeng L, Liu L, Liu FJ, et al. Prevalence and risk factors of fatty liver disease in Chengdu, Southwest China. Hepatobiliary Pancreat Dis Int 2009;8:377-82.
6. Bajaj S, Nigam P, Luthra A, Pandey RM, Kondal D, Bhatt SP, et al. A case-control study on insulin resistance, metabolic co-variates& prediction score in non-alcoholic fatty liver disease. Indian J Med Res 2009; 129:285-92.
7. Dassanayake AS, Kasturiratne A, Rajindrajith S, Kalubowila U, Chakrawarthy S, De Silva AP, et al. Prevalence and risk factors for non-alcoholic fatty liver disease among adults in an urban Sri Lankan population. J Gastroenterol Hepatol 2009;24:1284-8.
8. Mohan V, Farooq S, Deepa M, Ravikumar R, Pitchumoni CS. Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome. Diabetes Res Clin Pract 2009;84:84-91.
9. Zhou YJ, Li YY, Nie YQ, Ma JX, Lu LG, Shi SL, et al. prevalence of fatty liver disease and its risk factors in the population of South China. World J Gastroenterol 2007;13:
10. Chen CH, Huang MH, Yang JC, Nien CK, Yang CC, Yeh YH, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in an adult population of Taiwan: metabolic significance of non-alcoholic fatty liver disease in nonobese adults. J Clin Gastroenterol 2006;40:745-52.

11. Zelber-Sagi S, Nitnaz-Kaluski D, Halpern Z, Oren R. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. Liver Int 2006;26:856-63.

12. Park SH, Jeon WK, Kim SH, Kim JH, Park DI, Cho YK, et al. Prevalence and risk factors of non-alcoholic fatty liver disease among Korean adults. J Gastroenterol Hepatol 2006;21:138-43.

13. Jimba S, Nakagami T, Takahashi M, Wakamatsu T, Hirota Y, Iwamoto Y, et al. Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. Diabet Med 2005;22:1141-5.

14. Fan JG, Zhu J, Li XJ, Chen L, Li L, Dai F, et al. Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. J Hepatol 2005;43:508-14.

15. Singh SP, Nayak S, Swain M, Rout N, Mallik RN, Agrawal O, et al. Prevalence of non-alcoholic fatty liver disease in coastal eastern India: a preliminary ultrasonographic survey. Trop Gastroenterol 2004;25:76-9.

16. Omagari K, Kadokawa Y, Masuda Ji, Egawa I, Sawa T, Hazama H, et al. Fatty liver in non-alcoholic non-overweight Japanese adults: incidence and clinical characteristics. J Gastroenterol Hepatol 2002;17:1098-105.

17. Loomer R, Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol 2013;10:686-90.

18. El-Kader SM, El-Den Ashmawy EM. Non-alcoholic fatty liver disease: The diagnosis and management. World J Hepatol 2015;7:846-58.

19. Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, et al. Associations between serum 25-hydroxyvitamin D 3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2007;17:517-24.

20. Lee SM, Jun DW, Cho YK, Jang KS. Vitamin D deficiency in non-alcoholic fatty liver disease: The chicken or the egg? Clin Nutr 2017;36:191-97.

21. Barchetta I, Angelico F, Del Ben M, Baroni MG, Pozzilli P, Morini S, et al. Strong association between non-alcoholic fatty liver disease (NAFLD) and low 25 (OH) Vit D levels in an adult population with normal serum liver enzymes. BMC Med 2011;9:85.

22. Katz K, Brar PC, Parekh N, Liu Y-H, Weitzman M. Suspected non-alcoholic Fatty liver disease is not associated with vitamin D status in adolescents after adjustment for obesity. J Obes 2011;2010:496829.

23. Junn E, Han SH, Im JY, Yang Y, Cho EW, Um HD, et al. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. The J Immunol 2000;164:6287-295.

24. Urrutia-Pereira M, Solé D. Vitamin D deficiency in pregnancy and its impact on the fetus, the newborn and in childhood. Rev Paul Pediatr 2015;33:104-13.

25. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997;7:439-43.

26. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998;351:805-6.

27. Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. Am J Clin Nutr 2004;80:1706-9.

28. Scatarije JC, Scott WW, Donovan PJ, Siegelman SS, Sanders RC. Fatty infiltration of the liver: ultrasonographic and computed tomographic correlation. J Ultrasound Med 1984;3:9-14.

29. Cordeiro A, Pereira S, Saboya CJ, Ramalho A. Relationship between Nonalcoholic Fatty Liver Disease and Vitamin D Nutritional Status in Extreme Obesity. Can J Gastroenterol Hepatol 2017; 2017:9456897.

30. Jablonski KL, Jovanovich A, Holmen J, Targher G, McFann K, Kendrick J, et al. Low 25-hydroxyvitamin D level is independently associated with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2013; 23:792-98.

31. Eliades M, Spyrou E, Agrawal N, Lazo M, Brancati FL, Potter JJ, et al. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2013;38:246-54.

32. Kayaniyil S, Vieth R, Harris SB, Retnakaran R, Knight JA, Gerstein HC, et al. Association of 25 (OH) D and PTH with metabolic syndrome and its traditional and nontraditional components. J Clin Endocrinol Metab 2011;96:168-75.

33. Cabral MA, Borges CN, Maia JM, Aires CA, Bandeira F. Prevalence of vitamin D deficiency during the summer and its relationship with sun exposure and skin phenotype in elderly men living in the tropics. Clin Interv Aging 2013;8:1347-51.

34. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011;34:274-85.