

Association Between Serum Concentrations of 25-hydroxy Vitamin D3 and Severity of Liver Stiffness by Using Vibration-Controller Transient Elastography

Eskandar Hajiani, Pezhman Alavinejad, Saieed Mathinkhah, Zohreh Badiiee, Seyed Jalal Hashemi, Abdol Rahim Masjedizadeh, Ahmad Kadkhodae

Eskandar Hajiani, Pezhman Alavinejad, Seyed Jalal Hashemi, Abdol Rahim Masjedizadeh, Ahmad Kadkhodae, Research Institute for Infectious Diseases of Digestive System, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Eskandar Hajiani, Seyed Jalal Hashemi, Abdol Rahim Masjedizadeh, Associate Professor of Gastroenterology, Department of Internal Medicine, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Pezhman Alavinejad, Ahmad Kadkhodae, Assistant Professor of Gastroenterology, Department of Internal Medicine, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Saieed Mathinkhah, Fellowship of Gastroenterology, Department of Internal Medicine, School of Medicine, Ahvaz University of Medical Sciences, Ahvaz, Iran

Zohreh Badiiee, Associate professor, Department of Pediatrics, School of Medicine, and Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to: Pezhman Alavinejad, Research Institute for Infectious Diseases of Digestive System, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Email: palavinezhad@yahoo.com

Telephone: +989161115880 Fax: +98 611 3382342

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ABSTRACT

AIM: To evaluate the association between serum concentration of 25-hydroxy vitaminD3 [25(OH) D] and liver stiffness severity based on vibration- controller transient elastography (VCTE) in patients with nonalcoholic fatty liver disease (NAFLD).

METHOD: Sixty two patients with NAFLD enrolled the study from May 2012 to August 2013. The severity of liver stiffness evaluated by VCTE. The correlation between severity of liver stiffness and serum

levels of 25 (OH) D, total cholesterol, triglyceride, Fasting plasma glucose, ALT and AST were investigated.

RESULTS: There was no significant correlation between severity of liver stiffness and serum concentration of 25 (OH) D ($P=0.388$) while we found a significant correlation between serum AST level and severity of liver stiffness ($P=0.0001$).

CONCLUSION: There is no correlation between serum concentration of 25 (OH) D and severity of liver stiffness using VCTE.

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Key words: Nonalcoholic fatty liver disease; Vibration- controller transient elastography; 25-hydroxy vitaminD3

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), which includes a wide spectrum from simple steatosis (non-harmful accumulation of fat in the liver cells) to progressive nonalcoholic steatohepatitis (NASH) with or without cirrhosis and hepatocellular carcinoma, has become a worldwide public health concern^[1]. The prevalence of NAFLD ranges from 10 to 24% depending on the criteria used for specific diagnosis^[2]. It is one of the most common chronic liver diseases among general population and because of growing incidence of obesity and diabetes mellitus and decreasing incidence of viral hepatitis, it is anticipated that the incidence of NAFLD will proceed viral hepatitis in the future^[3,4].

The progression of NASH is not uniform and NASH with fibrosis promote faster than NASH without fibrosis. Recently, it is demonstrated that hepatocellular carcinoma (HCC) can occur not only in patients with NASH induced cirrhosis, but also in NASH patients without advanced fibrosis^[5].

Although, our knowledge about histopathologic characteristics of NASH is improving, the pathogenesis of NAFLD is not completely clarified. NAFLD in most patients is associated with metabolic risk factors including obesity, diabetes mellitus, and dyslipidemia. In addition, recent studies have shown the association between NAFLD and some other diseases including obstructive sleep apnea (OSA), colonic adenomas, hyperuricemia, hyperferritinemia, pancreatic steatosis, hypothyroidism and vitamin D deficiency^[6,7]. On the other hand, it has been documented that vitamin D deficiency has non-skeletal adverse effects on several organ systems. Some researches proposed low 25-hydroxy vitaminD3 [25(OH) D] concentrations may be associated with some cancers, type 2 diabetes Mellitus, metabolic syndrome, cardiovascular disease and insulin resistance^[8,9]. Some studies demonstrated a close relationship between 25(OH) D level, calcium hemostasis and cardiovascular disease^[10,11] while others showed impaired glucose tolerance, metabolic syndrome and type 2 diabetes mellitus to be more prevalent in individuals with low serum concentration of 25 (OH) D^[12,13,14]. Vitamin D can decrease FFA-induced insulin resistance in peripheral tissues and hepatocytes. As a result, low serum vitamin D may predispose liver to lipid accumulation and resulting NAFLD^[15].

Liver biopsy is the gold standard for diagnosis of broad range of NAFLD. However, in practical hepatology liver biopsy is not recommended routinely for diagnosis of NAFLD due to its risk of bleeding, expense, and absence of specific criteria to differentiate NAFLD entities^[16].

There are several methods for diagnosis of steatosis. Ultrasonography is the least expensive and easiest modality for diagnosing NAFLD. Recently, vibration- controller transient elastography (VCTE) has been offered for liver stiffness assessment. VCTE is considered to be a reliable technique for detection of advanced liver fibrosis and early liver cirrhosis^[17,18,19]. This study designed to evaluate the relationship between serum 25(OH) D concentrations and severity of liver stiffness based on VCTE findings.

MATERIALS AND METHODS

Overall we assessed 62 sequential patients with NAFLD who attended our hepatology clinic, affiliated to Ahvaz Jundishapur University of Medical Sciences from May 2012 to August 2013. The diagnosis of NAFLD was based on two criteria including liver ultrasonographic findings compatible with NAFLD reported by an experienced radiologist and serum alanine aminotransferase (ALT) more than 40 U/L. Exclusion criteria included any history of advanced chronic liver or renal disease, alcohol intake more than 20 g/day, viral hepatitis, autoimmune hepatitis, use of hepatotoxic drugs, receiving any medication that affecting vitamin D metabolism, history of hypothyroidism or Cushing syndrome, taking parenteral nutrition since 6 months ago, history of surgery on digestive tract such as gastroplasty or jejunioileal bypass. The research ethical committee of Ahvaz Jundishapur University of Medical Sciences approved the study and informed consent was obtained from the participants. We measured body weight and height and calculated body mass index (BMI) by dividing weight (in kilograms) by height (in square meter). Blood pressure was measured on 3 occasions by

experienced nurses. We were taking venous blood in the morning after at least 12 hours fasting and the following tests were performed by using standard laboratory methods: fasting blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipid profile including triglycerides, high density lipoprotein (HDL) cholesterol, total cholesterol, blood urea nitrogen (BUN), creatinine, thyroid function tests and 25(OH) D concentration. Low density lipoprotein (LDL) cholesterol was calculated by Fried Wald equation.

Liver ultrasonography was performed to evaluate the degree of steatosis by the same operator who was blinded to the study protocol. Liver steatosis was scored semi quantitatively on a scale of 0-3. All patients were undergone VCTE (FibroScan[®], Echosense, Paris, France) for liver stiffness assessment.

Our statistician calculated a sample size of 61 patients to achieve a statistically significant difference in liver stiffness score with a power of 80% and a *P* value <0.05. The subject characteristics were compared by student's *t* test for numerical data and chi-square or Fisher exact test for qualitative variables. We used SPSS software (version 21, SPSS Inc., Chicago, IL) for data analysis.

RESULTS

From May 2012 to August 2013, 178 patients were assessed for eligibility and finally 62 patients were included. The main reason of exclusion was refusal to perform VCTE. Ten patients excluded because of using Tamoxifen and methotrexate and 13 patients because of viral hepatitis.

Demographic characteristics of patients are shown in table 1. Thirty seven of 62 patients (59.7%) had low serum 25(OH) D concentrations (less than 20 nmol/L).

Table 1 Demographic characteristics of study population.

| Parameter | Mean | ± SD |
|---------------------------------|----------------------|------|
| age | 40.19 | 8.7 |
| Sex (m/f) | 25/37 (40% m, 60% f) | |
| BMI | 28.29 | 3.1 |
| triglyceride | 216.00 | 74.8 |
| Total cholesterol | 196.9 | 41.9 |
| aspartate aminotransferase(AST) | 45.4 | 25.1 |
| alanine aminotransferase(ALT) | 77.8 | 54.7 |
| fasting blood glucose | 103.5 | 25 |
| 25(OH)D | 20.6 | 13.5 |

There was no significant correlation between severity of liver stiffness and serum concentrations of 25(OH) D (*P*=0.388). Likewise, there were no significant correlation between severity of liver stiffness and patients age (*P*=0.562), ALT (*P*=0.77), FBS (*P*=0.064), total cholesterol (*P*=0.732) and TG (*P*=0.138). However, there was a strong correlation between severity of liver stiffness and serum concentration of AST (*P*=0.0001) (Table 2).

The mean (± SD) Liver stiffness score based on VCTE results were 5.94±2.51.

We categorized liver stiffness severity to 5 groups based on a previous study (20) which is shown in table 3.

DISCUSSION

Although about 56% of our NAFLD patients had marked deficiency of 25(OH) D, our study did not show any significant correlation between serum concentration of 25(OH) D and degree of liver stiffness according to VCTE.

Several studies have shown higher prevalence of vitamin D deficiency among NAFLD subjects compared with controls^[21,22,23,24].

Table 2 Correlation between FBS, AST, ALT, lipid profile, 25(OH) D concentration and liver stiffness.

| | FBS | AST | ALT | TG | TC | BMI | Age | 25(OH)D | Liver stiffness |
|---------------------|-------|--------|-------|--------|-------|-------|-------|---------|-----------------|
| Liver stiffness | | | | | | | | | |
| Pearson correlation | 0.060 | 0.515 | 0.038 | -0.192 | 0.046 | 0.213 | 0.075 | 0.112 | - |
| <i>p</i> | 0.643 | 0.0001 | 0.77 | 0.138 | 0.732 | 0.099 | 0.562 | 0.388 | |
| FBS | | | | | | | | | |
| Pearson correlation | - | | | -0.022 | 0.126 | | | 0.080 | 0.06 |
| <i>p</i> | | | | 0.867 | 0.335 | | | 0.535 | 0.064 |
| AST | | | | | | | | | |
| Pearson correlation | | - | 0.506 | | | | 0.151 | | 0.515 |
| <i>p</i> | | | 0.000 | | | | 0.243 | | 0.0001 |
| ALT | | | | | | | | | |
| Pearson correlation | | 0.506 | | | | | 0.324 | | 0.038 |
| <i>p</i> | | 0.000 | | | | | 0.010 | | 0.77 |
| TG | | | | | | | | | |
| Pearson correlation | 0.022 | | | - | 0.178 | | | 0.204 | -0.192 |
| <i>p</i> | 0.867 | | | | 0.170 | | | 0.114 | 0.138 |
| TC | | | | | | | | | |
| Pearson correlation | 0.126 | | 0.324 | 0.178 | - | | | -0.004 | 0.046 |
| <i>p</i> | 0.335 | | 0.010 | 0.170 | | | | 0.975 | 0.732 |
| Age | | | | | | | | | |
| Pearson correlation | | 0.151 | | | | | - | - | 0.075 |
| <i>p</i> | | 0.243 | | | | | | | 0.562 |
| 25(OH)D | | | | | | | | | |
| Pearson correlation | -0.08 | | | -0.204 | 0.004 | | | - | 0.112 |
| <i>p</i> | 0.535 | | | 0.114 | 0.975 | | | | 0.388 |

Table 3 The severity of liver stiffness.

| Liver stiffness score | Number of patients (%) |
|-----------------------|------------------------|
| F0 (less than 5.6) | 36 (41.6) |
| F1(5.7-6.65) | 9 (16.6) |
| F2 (6.66-8) | 4 (6.6) |
| F3 (8.1-17) | 9 (15) |
| F4 (more than 17.1) | 0 (0) |

Barchetta and colleagues investigated the association between hypovitaminosis D and presence of NAFLD in patients with various degree of insulin resistance and demonstrated that patients with NAFLD had reduced level of 25(OH) D compared to patients without NAFLD^[24]. They also showed the relationship between hypovitaminosis D and NAFLD to be independent of age, sex, triglycerides, HDL and glucose concentrations. However, their definition of NAFLD was based only on ultrasonographic findings and they did not confirmed NAFLD diagnosis with liver biopsy or VCTE. In addition, they did not clarify the season of serum 25(OH) D measurement and ignored the importance of sun exposure on serum concentration of 25(OH) D. It is important to consider this factor for interpretation of results^[24].

Targher and coworkers evaluated the association between serum concentration of 25(OH) D and liver histology in NAFLD patients and showed that in comparison to control group, NAFLD patients had a marked decrease in winter serum 25(OH) D concentrations, which was closely associated with histopathological features of NAFLD^[11]. Our basis for NAFLD diagnosis was VCTE and not liver biopsy. Although, the mean serum concentration of 25(OH) D in NAFLD patients in our study was 20.5 nmol/L which is very close to the figure 20.4 in Targher's study, we did not found any association between NAFLD and serum concentration of 25(OH) D. We speculated that this difference in results may be due to different geographic regions of two studies. Ahvaz city situated in a tropical region and sun exposure exists throughout the year. Therefore there is not seasonal variations of serum concentration of 25(OH) D. On the other hand, discrepancy of our results with results of two above studies may be due to differences in methodology. Barchetta and coworkers used only ultrasonography but we used VCTE and Targher and associates used liver biopsy for NAFLD diagnosis.

Some studies from Asia proposed that the association between vitamin D and NAFLD may be affected by ethnicity^[21,25]. Li and coworkers assessed the association between serum vitamin D concentration and NAFLD by using radioimmunoassay in a Chinese population from May to July. They did not find any positive correlation between serum vitamin D concentration and NAFLD^[26]. This could be explained by allele frequency of vitamin D receptor polymorphism between western and eastern people^[27].

We did not found any association between NAFLD and ALT, fasting blood glucose, serum triglyceride, total cholesterol, and 25 (OH) D concentration although there was a strong correlation between AST and liver stiffness which guide us to have more focus on the level of AST in evaluating patients. Similarly, Verma and colleagues evaluated predictive value of ALT level for NASH and NAFLD and demonstrated that the rate of advanced fibrosis was not different between normal and elevated ALT groups. However, they showed that NASH was significantly more common in patients with high ALT. They concluded that there is no cutoff point for ALT level to predict NASH and advanced fibrosis^[26].

Our study had some limitations including lack of a control group with normal VCTE and relatively small sample size. However, it is the first study that evaluated the correlation between serum concentration of vitamin D with NAFLD by using VCTE which is a noninvasive and reliable method for detection of liver injury.

CONCLUSION

Our results demonstrated that there is no correlation between serum concentrations of vitamin D with NAFLD based on VCTE results however further investigation in different geographic regions are needed to clarify this issue

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

REFERENCES

- 1 Liangpunsakul S, Chalasani N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? *J Clin Gastroenterol* 2003; **37(4)**: 340-3
- 2 Lizardi-Cervera J, Aguilar-Zapata D. Nonalcoholic fatty liver disease and its association with cardiovascular disease. *Ann Hepatol* 2009; **8(1)**: S40-3
- 3 Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43(S1)**: S99-S112
- 4 Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. Seminars in liver disease [Internet]. \copyright Thieme Medical Publishers; 2008 [cited 2014 Feb 7]. p. 339–50. Available from: <https://www.thieme-connect.com/ejournals/abstract/10.1055/s-0028-1091978>
- 5 Torres DM, Williams CD, Harrison SA. Features, diagnosis, and treatment of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc* 2012 Aug; **10(8)**: 837-58
- 6 Baranova A, Tran TP, Bircerdinc A, Younossi ZM. Systematic review: association of polycystic ovary syndrome with metabolic syndrome and non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2011 Apr; **33(7)**: 801-814
- 7 Lee YI, Lim Y-S, Park HS. Colorectal neoplasms in relation to non-alcoholic fatty liver disease in Korean women: a retrospective cohort study. *J Gastroenterol Hepatol* 2012 Jan; **27(1)**: 91-95
- 8 Boucher BJ. Inadequate vitamin D status: does it contribute to the disorders comprising syndrome “X”? *Br J Nutr* 1998 Apr; **79(4)**: 315-327
- 9 Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. *Int J Epidemiol* 1990 Sep; **19(3)**: 559-563
- 10 Anderson JL, May HT, Horne BD, Bair TL, Hall NL, Carlquist JF, Lappe DL, Mohlestein JB. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol* 2010 Oct 1; **106(7)**: 963-968
- 11 Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. *Atherosclerosis* 2009 Jul; **205(1)**: 255-260
- 12 Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007 Jun; **92(6)**: 2017-2029
- 13 Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes* 2008 Oct; **57(10)**: 2619-2625
- 14 Hyppönen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-I, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes* 2008 Feb; **57(2)**: 298-305
- 15 Zhou QG, Hou FF, Guo ZJ, Liang M, Wang GB, Zhang X. 1,25-Dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. *Diabetes Metab Res Rev* 2008 Sep; **24(6)**: 459-464
- 16 Adams LA, Feldstein AE. Non-invasive diagnosis of nonalcoholic fatty liver and nonalcoholic steatohepatitis. *J Dig Dis* 2011 Feb; **12(1)**: 10-16
- 17 Lee HY, Crawley S, Hokari R, Kwon S, Kim YS. Bile acid regulates MUC2 transcription in colon cancer cells via positive EGFR/PKC/Ras/ERK/CREB, PI3K/Akt/IkappaB/NF-kappaB and p38/MSK1/CREB pathways and negative JNK/c-Jun/AP-1 pathway. *Int J Oncol* 2010 Apr; **36(4)**: 941-953
- 18 Friedrich-Rust M, Ong M-F, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008 Apr; **134(4)**: 960-974
- 19 Hajiani E, Hashemi SJ, Masjedizadeh AR, Shayesteh AA, Alavi Nejad P, Kadkhodae A, Jalalvand K. Comparison of Liver Biopsy with Transient Elastography as a Non-invasive Method for Assessment of Liver Fibrosis. *Journal of Gastroenterology and Hepatology Research* 2014; **3(2)**: 1013-1016
- 20 Fung JYY. Measuring liver stiffness using transient elastography in the non-invasive assessment of chronic hepatitis B [Internet]. ResearchSpace@ Auckland; 2010 [cited 2014 Feb 7]. Available from: <http://researchspace.auckland.ac.nz/handle/2292/8347>
- 21 Dasarathy J, Periyalwar P, Allampati S, Bhinder V, Hawkins C, Brandt P, Khiyami A, McCullough AJ, Dasarathy S. Hypovitaminosis D is associated with increased whole body fat mass and greater severity of non-alcoholic fatty liver disease. *Liver Int Off J Int Assoc Study Liver* 2013 Sep 5;
- 22 Rhee E-J, Kim MK, Park SE, Park C-Y, Baek KH, Lee W-Y, Kang MI, Park SW, Kim SW, Oh KW. High serum vitamin D levels reduce the risk for nonalcoholic fatty liver disease in healthy men independent of metabolic syndrome. *Endocr J* 2013; **60(6)**:743-752
- 23 Kim D, Chung GE, Lim SH, Yim JY, Park MJ, Kim W, Kim YJ, Yoon JH, Lee HS. 1295 SERUM VITAMIN D IS INVERSELY ASSOCIATED WITH NONALCOHOLIC FATTY LIVER DISEASE IN GENERAL POPULATION. *J Hepatol* 2012; **56**: S511
- 24 Barchetta I, Angelico F, Del Ben M, Baroni MG, Pozzilli P, Morini S, Cavallo MG. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. *BMC Med* 2011; **9**: 85
- 25 Li L, Zhang L, Pan S, Wu X, Yin X. No significant association between vitamin D and nonalcoholic fatty liver disease in a Chinese population. *Dig Dis Sci* 2013 Aug; **58(8)**: 2376-2382
- 26 Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int Off J Int Assoc Study Liver* 2013 Oct; **33(9)**: 1398-1405
- 27 Fan L, Tu X, Zhu Y, Zhou L, Pfeiffer T, Feltens R, Stoecker W, Zhang R. Genetic association of vitamin D receptor polymorphisms with autoimmune hepatitis and primary biliary cirrhosis in the Chinese. *J Gastroenterol Hepatol* 2005 Feb; **20(2)**: 249-255

Peer reviewer: Munachika Enjoji, MD, PhD, Health Care Center, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.