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Vitamin D deficiency is associated with high levels of LDL cholesterol in NAFL children

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Abstract

Background: The association between vitamin D deficiency and children with obesity with non-alcoholic fatty liver disease (NAFL) has been described in several studies, but little is known about the exact mechanism which links Vitamin D to dyslipidemia and fatty liver disease in pediatric population.

Objective: Our aim of the present study was to investigate the relationship between Vitamin D (VD) serum levels and serum lipid profiles in a pediatric population with Non Alcoholic Fatty Liver Disease (NAFLD).

Methods: We enrolled 155 pediatric patients diagnosed with NAFLD. Patients were divided into two groups on the basis of the presence/absence of Vit D deficiency (VD-/VD+). Anthropometric (weight, height, waist circumference and BMI), parameters, laboratory values (e.g. triacylglycerols, cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose, insulin resistance and Vitamin D levels) and radiological (liver ultrasound for steatosis) parameters were taken.

Results: We found that BMI, blood pressure, TAG, LDL-C, insulin resistance and progressive liver steatosis were proportionally related to low VD serum levels. This showed that vitamin D deficiency may be associated with an increased risk of dyslipidemias: increase in serum of 10 ng/dL of VD was associated with decreases of 0.73 mg/dL in TAG and 0.98 mg/dL in LDL-C.

Conclusion: Vitamin D deficiency can be considered as a marker for elevated atherogenic lipoproteins and liver fibrosis taking part in the progression of pediatric NAFLD.

Abbreviation: ALT: Alanine-aminotransferases; AST: Aspartate-aminotransferases; BMI: Body Mass Index; TC: Total cholesterol; CPT-1: Carnitine palmitoyltransferase I; DBP: Diastolic blood pressure; GGT: Gamma-glutamyl-transpeptidase; HDL-C: High-density lipoprotein cholesterol; HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; HOMA-IR: Homeostatic model assessment of insulin resistance; INR: International Normalized Ratio; iSBT: ileal sodium/bile acid cotransporter; LDL-C: Low-density lipoprotein cholesterol; NAFLD: Non Alcoholic Fatty Liver Disease; NAS: NAFLD Activity Score; OGTT: Oral glucose tolerance test; SBP: Systolic blood pressure; SD: Standard Deviation; SREBP-1c: Sterol regulatory element-binding protein; TAG: Triacylglycerols; VD: Vitamin D; WC: Waist circumference; WHO: World Health Organization.

Introduction

Vitamin D is a steroid hormone involved in several physiological processes alongside bone metabolism. The predominant form of circulating vitamin D is 25-hydroxyvitamin D [25 (OH) D] which is hydroxylated by the liver both after ingestion or endogenous synthesis [1].

The importance of Vitamin D is illustrated by the diffuse expression of vitamin D receptor and its deficiency is associated with several diseases, including intestinal inflammatory diseases, infectious diseases and cardiovascular disorders [2,3].

Previous studies suggested that vitamin D and its derivatives might play a role in the regulation of cholesterol biosynthesis by inhibiting in vitro, in different cell culture lines, HMG-CoA enzyme reductase activity and thus cholesterol synthesis [4]. Over the last ten years, the study of Vitamin D Receptor has provided new insights regarding the vitamin D function, besides its role in bone metabolism. Cho, et al. suggested that increasing intestinal calcium absorption may reduce synthesis and secretion of hepatic TAG [5,6]. Vitamin D may inhibit synthesis and secretion of TAG through stimulating intestinal calcium assimilation, and the calcium may promote cholesterol conversion in bile acids and this reduces serum cholesterol levels. For this reason, the

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serum levels of LDL-C could be reduced by the decreased absorption of fat, particularly saturated fatty acids [7].

Moreover, vitamin D is known to stimulate the production of two orthologous fibroblast growth factors, FGF15/19 by intestinal epithelium, which alter biliary acid homeostasis in mice, but how FGF15/19 change in response to vitamin D supplementation can affect cholesterol, the precursor of bile acids, still needs to be studied [8].

To date, the association between vitamin D deficiency and children with obesity with non-alcoholic fatty liver disease (NAFL) has been described in several studies. In one study, the reduction of NAFLD Activity Score (NAS), triacylglycerols and HOMA-IR was demonstrated only in children treated with omega3 and Vitamin D [9]. Moreover, a recent review highlights that 25-hydroxivitamin D is directly associated with HDL-C and inversely related to total cholesterol, LDL and TAG [10]. Little is known about the exact mechanism which links Vitamin D to dyslipidemia and fatty liver disease in pediatric population. The main aim of the present study was to investigate the relationship Vitamin D status and lipids profile in NAFL in children.

Material and Methods

This study was approved by the Institutional Review Board (IRB) of "Bambino Gesu' Children Hospital", Rome, Italy, number of protocol 965B/RA.

We enrolled 155 consecutive children and adolescents with obesity/overweight (age 10 to 17 years) referred to Hepatometabolic Unit of the "Bambino Gesù" Children's Hospital from March 2016 to February 2017, to be evaluated for NAFL.

The diagnosis of NAFL was made on the presence of fatty liver upon ultrasound examination with or without hypertransaminasemia, after exclusion of other causes of hepatic steatosis (autoimmune hepatitis, viral and metabolic diseases, Wilson disease and/or genetic disorders, drugs). Patients with obesity secondary to genetic diseases, endocrinological or metabolic disorders were not included. We also excluded individuals who had supplementation of vitamin D or vitamin D analogues and any drugs that could have affected calcium and/or phosphorus metabolism.

All patients underwent anthropomorphic measurements including weight, height and waist circumference using standardized methods. The body mass index (BMI) and its standard deviation score (z-BMI) were calculated [11].

Blood samples were taken for triacylglycerols (TAG), cholesterol (C), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C); Apolipoprotein A1 (ApoA) and Apolipoprotein B (ApoB); liver function tests: aspartate- (AST) and alanine (ALT)- aminotransferases, gamma-glutamyl-transpeptidase (GGT) and international normalized ratio (INR); fasting plasma glucose and insulin in all patients. Moreover, oral glucose tolerance tests (OGTT) were performed according to the recommendations of the World Health Organization (WHO) [12]. Since we know that the "Homeostasis model assessment of insulin resistance" (HOMA-IR) is more reliable than fasting glucose/insulin ratio and quantitative insulin sensitivity check index (QUICKI), we used the first (HOMA-IR = insulin0 (μIU/ml) * glucose0 (mg/dl))/405) for assessing insulin resistance among children and adolescents with obesity. An HOMA-IR cut-off value of >2.5 was considered as index of insulin resistance [13,14].

In all patients, serum 25-hydroxyvitamin D [25(OH) D, vitamin D] concentration was measured by radioimmunoassay (IDS Immunodiagnostics, IDS Limited, Tyne and Wear, UK). Patients were categorized as having either several low vitamin D levels (<20 ng/mL), or normal vitamin D levels (≥20 ng/mL), cut-off limits were established according to Italian Consensus of 2015 and other studies [15-18].

All patients underwent abdominal ultrasound scan performed by a single experienced radiologist, who was blinded to the health conditions of the patients, using an Acuson Sequoia C512 scanner equipped with a 15L8 transducer (Universal Diagnostic Solutions, Oceanside, CA, USA) [19,20]. Liver steatosis was defined according to the following criteria: high-level echoes arising from the hepatic parenchyma and liver-kidney difference in echo amplitude. Steatosis was defined with semi quantitative scale of 0–6 : 0, absent; 1, 2 mild; 3, 4 moderate; and 5, 6 severe [21].

Among the study cohort, 62 patients (31 VitD$^+$, 31 VitD-) underwent also a liver biopsy (LB), according to position paper of ESPGHAN [20].

Echo-guided liver biopsy was performed using an automatic core biopsy device (Biopince, Amedic, Sweden) with an 18-G or 16-G needle, under general anesthesia [9]. The histological features of steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2) were combined in the NAFLD activity score (NAS), ranging from 0 to 8 using the criteria of NAFLD Clinical Research Network [22]. A single experienced pathologist evaluated liver biopsies.

Determination of total monthly hours of sunlight

The mean hours of sunshine was determined using the "Italian atlas of solar radiation" from the ENEA center (http://www.solaritaly.enea.it). The formula for estimating mean hours of sunlight was: % Sunshine x [(Clear days x 0.85) + (Partly Cloudy days x 0.45) + (Cloudy day x 0.10) x 24]

Sunshine % = the percentage of the daylight hours for Rome during that month;

Clear days= defined as 70%-100% of sunshine; was used for the mean value of 85% or 0.85 in the formula;

Cloudy days = defined as 30%-60% of sunshine; was used for the mean value of 45% or 0.45 in the formula;

Cloudy Days= defined as 0-20% of sunshine; was used for the mean value of 10% or 0.10 in the formula [9].

Theory/calculation

Data were analyzed using STATISTICA (version 2010, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD). Data distribution was checked for normality by the Kolmogorov-Smirnov test. Comparisons between the groups were done using the Student's t-test and the ANOVA, as appropriate. Difference between proportions were tested using the Chi-square test. Univariate correlations were investigated with Pearson's correlation. Multivariable regression analysis was also used to test the independence of associations between vitamin D concentrations as the key exposure and laboratory characteristics after adjusting for BMI.

Results

Between March 2016 and February 2017, 261 patients were screened and 155 of these with NAFLD were enrolled. The patients were enrolled with similar proportions recruited during the winter (65/155, 42%) and spring/summer (90/155, 58%) months.

All patients were obese/overweight (BMI 29.45±6.65). Based on the finding of vitamin D values, the patients were divided in two groups: patients with deficit of Vitamin D (<20 ng/dL) VD^-, 83 patients (53.5%) and patients with normal Vitamin (D ≥ 20 ng/dL) VD^+ 72 patients (46.5%),

In VD- group, 39 patients (46.9%) have severe steatosis, 24 (28.9 %) moderate and 20 (24.2%) the mild steatosis, while in VD^+ group 10 (14.8%) patients have severe, 31 (43.1%) moderate and 31 (43.1%) mild steatosis.

Anthropometric, clinical and laboratory parameters

Table 1 shows the anthropometric and laboratory characteristics for two groups: VD^- and VD^+.

In VD^- group the BMI (31.77 kg/mq *vs.* 27 kg/mq, p=0.01), z-BMI (2.16 *vs* 1.76, p=0.02) and WC (95,06 cm *vs.* 87.31, p=0.01) were significantly higher with respect to VD^+ ($0.01 \leq p < 0.02$). There was no significant difference for gender (p=0.057).

Moreover, the SBP (115 mmHg *vs.* 109 mmHg) and DBP (66.7 mmHg *vs.* 61.4 mmHg) were elevated in this group ($p<0.05$).

Laboratory tests showed that in VD^- groups the values of insulin (25.6 mU/L *vs.* 16.5 mU/L), HOMA-IR (5.2 *vs.* 3.2) and LDL-cholesterol (120.3 mg/dl *vs.* 98.2 mg/dl) were significantly higher, while the HDL-cholesterol (42.6 mg/dl *vs.* 48.8 mg/dl) significantly lower with respect to VD^+. Moreover, ApoA1, ApoB, calcium and phosphorus values were not significantly different between VD^+/VD^- groups.

These differences were confirmed with the Pearson's correlation. Plasma vitamin D levels were negatively associated to BMI (rho=-0.43; p=0.02), LDL-C (rho=-0.42; p<0.001) and fasting insulin (rho= 0.35, p=0.02) and HOMA-IR (rho=0.34, p=0.04).

The association between histological damage and Vitamin D levels

Only 62 patients, according to ESPGHAN [20], underwent a LB, 31 from $VitD^+$ group and 31 from $VitD^-$ group. NASH was diagnosed (NAS ≥ 5) in 8 (25.8%) patients from $VitD^-$ and in 3 (9.6%) patients from $VitD^+$, p<0.05 [Brunt NAS score].

The association between serum Vitamin D concentrations and lipids

In VD- group the 67 (80.7%) patients showed the LDL-C values >95th percentile, such as 22 (26.5%) with TAG >95th percentile and 16 (19.2%) with HDL <5th percentile for age, while in VD^+ group: 40 (55.5%) children had elevated LDL-C, 12 (16.6%) had elevated TAG and 8 (11.1%) had low HDL values.

Moreover, the multiple regression analysis was used to assess the associations between VD concentrations and lipids (Table 2). In our children, the VD concentrations were negatively associated with TAG and LDL-C after adjusting for BMI. Increase in serum of 10 ng/dL of VD was associated with decreases of 0.73 mg/dL in TAG and 0.98 mg/dL in LDL-C.

Table 1. Anthropometric, clinical and laboratory parameters. Differences were considered statistically significant at $p \leq 0.05$

(155, 85 M)	All (155)	Vitamin D <20 ng/ml (83)	Vitamin D >20 ng/ml (72)	p
Age (years)	12.81 (3.14)	13.67 (3.73)	12.19 (2.72)	0.10
Weight (mean ± SD)	75.7 (15.10)	81.19 (25.13)	71.18 (19.70)	0.02
BMI, Kg/mq (mean ± SD)	29.45 (6.65)	31.77 (7.1)	27.07 (4.77)	0.01
WC, cm (mean ± SD)	97.38 (14.64)	94.06 (15.74)	87.31 (14.66)	0.01
AST, UI/L (mean ± SD)	28.55 (12.14)	28.64 (13.96)	28.44 (9.75)	0.91
ALT, UI/L (mean ± SD)	34.91 (28.41)	36.13 (28.66)	33.5 (28.23)	0.56
Uric Acid, mg/dl (mean ± SD)	6.05 (4.17)	6.55 (2.44)	5.45 (1.14)	0.11
PCR, mg/dl (mean ± SD)	0.51 (0.41)	0.58 (0.61)	0.41 (0.36)	0.12
TC, mg/dl (mean ± SD)	161.71 (32.4)	178.09 (34.67)	159.98 (33.02)	0.14
LDL-C, mg/dl (mean ± SD)	109.21 (33.16)	120.32 (32.97)	98.23 (28.72)	<0.001
HDL-C, mg/dl (mean ± SD)	45.52 (17.78)	42.62 (12.78)	48.81 (21.75)	0.03
TAG, mg/dl (mean ± SD)	108.75 (54.18)	118.67 (56.24)	105.32 (54.12)	0.16
Apo-B, mg/dl (mean ± SD)	89.66 (53.83)	95.44 (49.65)	86.45 (43.87)	0.10
Apo-A1, mg/dl (mean ± SD)	118.21 (39.55)	117.86 (34.64)	119.32 (42.74)	0.76
Calcium, mg/dl (mean ± SD)	9.10 (1.55)	8.42 (1.53)	9.71 (1.90)	0.11
Phosphorus mg/dl (mean ± SD)	4.21 (2.51)	3.91 (1.92)	4.53 (1.64)	0.57
Fasting Glucose, mg/dl (mean ± SD)	80.97 (10.52)	82.72 (9.37)	80.22 (11.66)	0.41
Fasting Insulin, mU/L (mean ± SD)	21.12 (13.73)	25.67 (16.38)	16.51 (8.23)	<0.001
HOMA-IR (mean ± SD)	4.24 (2.93)	5.20 (3.47)	3.28 (1.85)	<0.001
SBP mmHg (mean ± SD)	107 (11.7)	115.36 (14.06)	109.53 (13.97)	0.03
DBP mmHg (mean± SD)	55.75 (9.47)	66.70 (13.24)	61.44 (10.84)	0.02
Vitamin D, ng/ml (mean ± SD)	20.27 (6.85)	13.86 (3.89)	25.43 (3.38)	<0.001

Table 2. The association between serum Vitamin D levels and serum after adjusting for age and BMI.

Variables	ß Coefficient	P
TC	0.02649	0.30
LDL-C	-0.9839	<0.0001
HDL-C	0.07366	0.84
TAG	-0.7345	0.001

β coefficient is a standardized in multiple linear regression analysis

Table 3. Odds ratio of dyslipidemia by serum Vitamin D levels. The OR and p-value for the prevalence of dyslipidemias between the two levels of serum Vitamin D were analyzed using logistic regression after adjustment for BMI. Data are presented as OR (95% CI)

	Vitamin D	Vitamin D >20 mg/dl	
		Odds ratio (95% CI)	p
Elevated TAG	1.00	0.91 (0.65 to 0.96)	0.04
Reduced HDL-C	1.00	0.78 (0.67 to 0.95)	0.03
Elevated LDL-C	1.00	0.56 (0.35 to 0.83)	0.001

The association between the dyslipidemias and Vitamin D levels

Adjusted OR for BMI and dyslipidemias by serum of Vitamin D levels were presented in Table 3. After adjusting for BMI, the OR for the elevated TAG (>95th percentile), reduced HDL-C (<5th percentile) and elevated LDL-C (>95th percentile) decreased significantly in VD^{+} compared with VD^{-} (Table 3).

Discussion

To the best of our knowledge, this is the first pediatric study evaluating the association between low vitamin D and dyslipidemia in patients with NAFL.

Vitamin D deficiency has been associated with a number of conditions including NAFL [23,24]. In a recent meta-analysis, it was shown that patients with NAFL were 1.26 times more likely to present a vitamin D deficiency than controls, also considering the major metabolic complications associated with NAFL [25,26].

Also, in our study we have shown that 53.5% of our children with NAFL had a vitamin D deficiency. They also have a more severe ultrasound picture of steatosis than patients with normal vitamin D. In fact, in the VD^{-} group 75.3% of the population had a moderate-severe steatosis degree, clearly higher than the VD^{+} group (57.9%). Furthermore, we have shown that in patients undergoing liver biopsy, NASH was more present in the vitamin D deficient group.

Based on these findings, recent studies have suggested that vitamin D deficiency may also be associated with the increased risk of lipid dyslipidemia in patients with NAFL [27].

Dyslipidemia is characterized by increases in LDL-C, increased TAG and decreases in HDL-C and cardiovascular diseases have been well defined as risk factors. In several populations it has been shown that high cholesterol values (TC and LDL-C) are associated with low levels of vitamin D, reinforcing the hypothesis that associations between vitamin D levels and serum lipid profiles exist between different populations and maintenance of vitamin D sufficiency seems to have a beneficial effect on serum lipids [28].

In our study we have shown that in a population of children with NAFL the 80.7% and 26.5% of the VD- showed LDL-C and TAG> 95th percentile values, compared with 55.5% and 11.1% of the VD^{+} and that the vitamin D concentration was inversely correlated with LDL-C values (rho= -0.42, p <0.001) as well as to the BMI (rho = -0.43, p = 0.02).

These results can be theoretically explained by the common biosynthetic way that cholesterol and vitamin D share in hepatocytes. Therefore, a biosynthesis of the upregulated cholesterol can lead to a reduction in vitamin D biosynthesis, explaining the inverse association between the plasma levels of atherogenic lipoproteins (LDL-C) and vitamin D, which is widely demonstrated in our study.

As regards the inverse association with the values of TAG, in several studies it has been shown that vitamin D can inhibit the synthesis and secretion of TAG due to the increase in intestinal calcium levels which induce a reduced absorption of fatty acids and therefore a reduced substrate for the synthesis of triacylglycerols [29]. However, in studies conducted on animal models, it appears that vitamin D may suppress the hepatic level of binding protein 1 of the sterol regulatory elements (SREBP-1c) to contain the synthesis of TAG and induce carnitine palmitoyltransferase I (CPT-1) promote energy expenditure in order to maintain the balanced levels of hepatic triacylglycerols [30,31]. Furthermore, it must be considered that the absorption of intestinal lipids also depends on the availability of bile salts, which are synthesized in hepatocytes from cholesterol. In a recent study on animal models, vitamin D deficiency was related to poor retention of bile acids in the liver in line with the reduction of the cotransport system (iSBT) of the sodium-dependent apical apparatus. Biliary acid obstruction was related to increased expression of SREBP-1c hepatitis and fatty acid synthesis, suggesting that vitamin D deficiency may increase endogenous fatty acid synthesis in NASH via compromised enterohepatic circulation. These results suggest that VD deficiency reduces iSBT expression, resulting in low bile acid content and increased lipogenesis and liver inflammation.

Finally, to reinforce what was previously stated, it seems that an increase in serum of 10 ng/dL of VD was associated with decreases of 0.73 mg/dL in TAG and 0.98 mg/dL in LDL-C in our population NAFL.

Some limitations must be recognized: the study was conducted in a single center which limits the overall application of the results to the whole country. Another limitation is that the associations of vitamin D and lipids are probably influenced by parathyroid hormone levels that have not been consistently evaluated in all patients. Furthermore, our population consists of patients with obesity and/or overweight who, as is known, have lower vitamin D values than the general population.

In conclusion, although we are currently unable to understand the specific etiopathogenic mechanism that links vitamin D to the genesis of dyslipidemia and the progression of fatty liver disease, our results confirm the hypothesis that even in the pediatric population affected by NAFL, vitamin D deficiency is related to atherogenic dyslipidemia, which is one of the causes of NAFL and its progression towards non-alcoholic steatohepatitis. However, large randomized trials are needed to better understand the relationship between vitamin D profiles and serum lipids, in order to prevent not only the NAFL but also cardiovascular diseases, improving the quality of life of young patients and reducing health costs of Western countries.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The reporting of this work is compliant with STROBE2 guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The study protocol was approved by the Ethical Committee of Bambino Gesù Children's Hospital and written informed consent was obtained from the parents of the children.

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