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ORIGINAL RESEARCH

Medicine Science International Medical Journal

Medicine Science 2019;8(4):908-12

Is vitamin D an important factor in hepatosteatosis in childhood obesity?

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Received 27 August 2019; Accepted 30 October 2019
Available online 10.12.2019 with doi:10.5455/medscience.2019.08.9107

Abstract
Childhood obesity is one of the most serious public health problems with obesity-related complications such as hepatosteatosis or type 2 diabetes occurring during early childhood. The aim of this study was to examine the relationship between 25-hydroxy vitamin D levels and obesity with hepatosteatosis (HS) in children. 128 obese children participated in this study. Hepatosteatosis was diagnosed and graded using ultrasonography in all patients. Serum levels of 25-hydroxy vitamin D, calcium, phosphate, alkaline phosphatase (ALP), parathormone (PTH), lipid, glucose and insulin were also measured. The data was analyzed across two groups of obese children – those with hepatosteatosis and those without hepatosteatosis. Forty-two percent of the study group were male. The mean age of the subjects was 12.1±3.1 years (range 4-18 years). Hepatosteatosis was identified in 39% of children (n: 50). A high prevalence (122/128 cases, 95%) of either 25-hydroxy vitamin D deficiency or insufficiency was determined. However, there was no statistically significant association between 25-hydroxy vitamin D levels and hepatosteatosis. Uric acid, alanine aminotransferase (ALT) and triglyceride levels were significantly higher in the HS group compared to non-HS group. There is a high prevalence of 25-hydroxy vitamin D deficiency and insufficiency among children with hepatosteatosis. However, in this study, no association was observed between 25-hydroxy vitamin D deficiency and hepatosteatosis.

Keywords: Childhood, Obesity, Hepatosteatosis, 25 hydroxy vitamin D

Introduction

Obesity is one of the most serious children's health problems and one that is extremely difficult to overcome. Furthermore, childhood obesity carries a significant risk factor for obesity later on in adulthood. Obesity development is not only linked to environmental factors – genetics, epigenetic factors and ethnicity all have roles to play here. Obesity is defined as an increase in body fat mass, yet, it is not simply a matter of weight gain. Other more serious complications can arise – one such example is non-alcoholic fatty liver disease (NAFLD). Thus, the main aim should be on preventing obesity before further complications have a chance to occur [1].

Non-alcoholic fatty liver disease is the most common form of chronic liver damage in obese children in Western populations and has a prevalence of 60-80% [2-5]. Fatty liver is a manifestation of metabolic syndrome in the liver. NAFLD manifests itself in a wide spectrum of ways, from simple fatty liver to hepatosteatosis, cirrhosis and hepatocellular carcinoma. Hepatosteatosis is generally asymptomatic and is used to define fatty liver without inflammation. Liver enzymes can be tested to determine hepatic damage in the obese cases. While hepatic ultrasound and magnetic resonance imaging can be used in other selected cases. Patients with elevated liver enzymes should be evaluated for hepatitis, autoimmune disease, Wilson's disease and alpha-1 antitrypsin deficiency.

After seeing the importance of 25-hydroxy vitamin D in autoimmune and inflammation processes; an evaluation of 25-hydroxy vitamin D's place in cases of metabolic syndrome, hypertension, insulin resistance and NAFLD has become necessary. Inflammation of adipose tissue and the liver are both potential effects of vitamin D deficiency. Moreover, spontaneous liver damage and hepatic fibrosis have been reported in vitamin D receptor knock-out rats [6].

The aim of this study was to compare 25-hydroxy vitamin D levels in obese children with fatty and non-fatty livers, and also to examine the relationship between 25-hydroxy vitamin D levels and hepatosteatosis.

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Material and Methods

This retrospective study was performed in the Samsun Gynecology and Child Health and Diseases Hospital between January and March 2015. The approval of the local ethics committee was obtained prior to commencing the study. Informed consent forms were signed by the parents of the children included in the study.

The height and weight of the children included in the study were measured. These measurements were taken by the same person using the same height and weight scales after the children's outer garments and shoes were removed. The children's heights were measured using a scale sensitive to 1 mm difference (Seca 703 accurate to 100gr; SecaGmbH&Co KG, Hamburg, Germany) with the children's bare feet placed straight on the ground and with heels touching. With the back of the head, upper back, hips and shoulders touching the device and the head held upright and looking ahead, height was measured by lowering and pressing the measuring rod on the hair. Weight was measured with each participant stepping on the device with bare feet and wearing only light clothing. The ideal body weight percentile and the Body Mass Index (weight (kg)/height squared (m2)) was calculated for each child based on their weight and height measurements. The Body Mass Index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. A BMI value in the 85-95th percentiles for age and gender was classified as overweight, with children whose BMI was above the 95th percentile according to age and gender were classified as obese. We excluded patients using medicine at the time or patients with elevated liver enzymes diagnosed as hepatitis, autoimmune disease, Wilson's disease and alpha-1 antitrypsin deficiency.

Venous blood samples were taken from all children following a 12-h overnight fasting for the measurement of fasting blood glucose, fasting insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) calcium, phosphate, parathormone (PTH) and cholesterol levels.

Triglyceride, HDL, cholesterol and glucose levels were analyzed using a Roche kit and autoanalyzer. Insulin and PTH levels were measured using a Bio-DPC kit and Immulite 2000 instrument (following the chemiluminescence method). Dyslipidemia was diagnosed where the patients had TG≥ 150 or LDL≥ 130 mg/dl, in accordance with the National Lipid Association 2014.

Insulin resistance was evaluated using the homeostasis model assessment of insulin resistance method (HOMA-IR) with the following formula [HOMA-IR: fasting glucose (mmol/L) x fasting insulin (μu/L) /22.5]. Insulin resistance is defined in children where the HOMA-IR level >3.16. Furthermore, insulin resistance was defined (according to HOMA-IR) as 2.67 in boys and 2.22 in girls in the prepubertal period and 5.22 in boys and 3.82 in girls [3]in the pubertal period.

Based on reference levels of ALT kits used in our hospital laboratory, ALT levels > 22 U/L in females and > 25 U/L in males were regarded as elevated liver enzymes [4].

All abdominal ultrasound examinations were performed by the same radiologist. A Toshiba Xario US instrument and 3.5 MHz convex probe were used for hepatobiliary ultrasonography. Steatosis of the liver was graded as follows: Grade 0: Normal parenchymal liver echogenicity; Grade I: Increased liver echogenicity with no haziness of vessel walls. Grade II: Increased liver echogenicity with haziness of vessel walls. Grade III: Increased liver echogenicity leading to the loss of normal contrast between the liver and the diaphragm. Patients were divided into two sub-groups based on the presence – or not - of hepatosteatosis. (Group 1: hepatosteatosis, Group 2 : Non-hepatosteatosis).

25-hydroxy vitamin D levels were measured using the immunochemiluminescence method. All samples were taken in the same season. 25-hydroxy vitamin D levels >20 ng/ml were seen to be indicative of vitamin D sufficiency and 25-hydroxy vitamin D levels <20 ng/ml as vitamin D deficiency and insufficiency [5] .

The statistical analysis was performed using the Statistical Package for the Social Sciences 22.0 (SPSS, Inc. Chicago IL, USA, Microsoft) software. Values were expressed as mean±standard deviation (minimum-maximum), frequency distribution and percentage. Pearson's chi-square test was used for the analysis of categorical variables. Normal distribution of variables was examined using visual tests (histogram and probability graphics) along with analytical methods (the Kolmogorov-Smirnov test). For contrary distributed variables, the Mann Whitney U test was used to determine statistical significance between two independent groups. P-values <0.05 were considered to indicate statistically significant differences.

Results

128 obese children (66 males and 62 females), aged between 4 and 18 years (mean age 12.1±3.1 years) participated in this study. Sixty-four patients (50%) were aged 4-12 years and the other 64 (50%) were aged 12-18 years. All of the children were examined using abdominal ultrasonography and hepatosteatosis was determined in 50 cases (39%). 121 (95%) of the cases enrolled in this study had 25-hydroxy vitamin D levels below 20 ng/ml.

There were significant differences between groups 1 and 2 in terms of BMI, weight SDS, LDL, ALT, uric acid and HOMA-IR values ($p<0.05$). However, no statistically significant difference was determined between the groups in terms of 25-hydroxy vitamin D levels. Mean 25-hydroxy vitamin D levels were 12.0±5.6 ng/ml in Group 1 and 11.2±5.7 ng/ml in Group 2 (Tables I-II).

No significant differences were determined between the two groups in terms of calcium, phosphate, alkaline phosphatase or parathormone (Ca, P, ALP or PTH) values. Forty-one patients had grade 1 hepatosteatosis, and nine had grade 2 hepatosteatosis. There was no significant difference in 25-hydroxy vitamin D levels between grade 1 and grade 2 hepatosteatosis.

Only 11 patients had 25-hydroxy vitamin D levels above 20 ng/ml. The hepatosteatosis ratio in cases with HOMA-IR values above 3.16 (n:66) or below 3.16 (n:62) were 48% (n:32) and 29% (n:18) respectively. 39% of patients had IR according to classification

related to Turkish ethnicity and there was no difference in the two groups for age, Homa-IR, BMI SDS , LDL, TG, Ca, 25-hydroxy vitamin D, PTH, üric asid (Table IV).

Hepatosteatosis frequency was 60% in females with ALT values >22 U/L and in males with ALT values >25 U/L (23/28). Hepatosteatosis frequency was 30% (27/88) in cases with ALT values below these figures (Table III). Only 13 patients with HS had ALT <25 U/L.

Using the Chi-square test, there was no relationship between a high ALT level (22 for girls, 25 I/U for boys) and steatosis in this study.

The lipid levels of 126 patients were evaluated and dyslipidemia was found in 15 (15/42, 35%) male and 24 (24/84 28%) female patients. 31% of patients had hyperlipidemia in this study. 20 patients had both HS and dyslipidemia and 19 patients had dyslipidemia but no HS. In our results, the lipid levels were not significantly different in the two groups based on steatosis. (p:0.08).

Table 1. The demographic and laboratory findings in two groups

	Hepatostaetosis n 50	Nonhepatosteatosis n 78	p-Value
Age (years)	12.0 ±2.8	11.7 ±3.2	0.040
Weightn(kg)	80 ±25	64± 20	0.01
BMI SDS	2.9± 0.8	2.5±0.6	0.01
HOMA-IR	4.8 ± 2.9	3.7 ±2.9	0.057
AST (U/L)	23.5± 9.3	20± 5.9	0.065
ALT(U/L)	28.0 ±20	18.0 ±9.0	0.01
D vit (ng/ml)	11.2± 5.7	12.5± 5.6	0.20
Ca (mg/dl)	9.9± 0.43	9.9 ±0.33	0.800
P (mg/dl)	4.5± 0.56	4.5 ±0.59	0.600
ALP(U/L)	215.0 ±99	216.0± 98	0.900
PTH(pg/ml)	44.0 ±18	46.0 ±33	0.73
TG (mg/dl)	135.0± 78	103.0 ±41	0.011
LDL (mg/dl)	102.0± 30	100.0 ±28	0.66
HDL (mg/dl)	43.7± 9	46.0 ±11	0.200
Uric acid (mg/dl)	5.4 ±1.3	4.9 ±1	0.018

Body mass Index (BMI), ,Homeostatic Model of Assessment-Insulin Resistance (Homa-IR), Triglycerides (TG), High density lipoprotein (HDL), Low density lipoprotein , (LDL) , Fasting blood glucose (FBG), Aspartate Aminotransferase (AST), Alanin Aminotransferase (ALT), Calsium (Ca), Phosphour (P), Alkalen phosphatase (ALP) Parathyroid hormone (PTH)

Table 2. Patient's demographic and laboratory data according to homa-IR >3.16 or <3.16

	HomaIR>3.16	HomaIR<3.16	p
Age (age)	13 .0±2,6	11.3 ±3.1	0,01
BMI	31.8 ±6	28.5 ±4.5	0.01
D vit(ng/ml)	11.2 ±5.7	12.0 ±5.6	0.500
Ca(mg/dl)	9.9 ±0.43	9.9± 0.39	0.500
P(mg/dl)	4.4± 0.5	4.5 ±0.59	0.200
ALP (U/L)	204.0 ±106.0	227.0 ±88.0	0.100
TG(mg/dl)	131.3 ±73	99 ±40	0.03
LDL(mg/dl)	100.3± 28	102.8 ± 29	0.600
HDL(mg/dl)	42.8 ±9.6	47.6± 10.9	0.011
Uric asid(mg/dl)	5.4 ±1.2	4.8 ±1,13	0.12
AST(U/L)	21.0 ±8.6	22.0 ±6.1	0.58
ALT(U/L)	23.8 ±17.8	21.3 ±11.9	0.37

Body mass Index (BMI), ,Homeostatic Model of Assessment-Insulin Resistance (Homa-IR), Triglycerides (TG), High density lipoprotein (HDL), Low density lipoprotein , (LDL) , Fasting blood glucose (FBG), Aspartate Aminotransferase (AST), Alanin Aminotransferase (ALT), Calsium (Ca), Phosphour (P), Alkalen phosphatase (ALP) Parathyroid hormone (PTH)

Table 3. The demographic and laboratory findings in two groups

	ALT>22(girls)/25 (boys) (n 38)	ALT<22(girls)/ 25(boys) (n 87)	p-Value
Age(years)	12.2 ±3.3	12.1± 2.9	0.88
D vit (ng/ml)	12.6± 5.3	11.6 ±5.7	0.39
Ca(mg/dl)	10 ±0.4	9.9± 0.39	0.11
P(mg/dl)	4.8± 0.5	4.5 ±0.5	0.03
ALP (U/L)	270 ±124	198.0 ±81.0	0.017
AST(U/L)	29.0 ±8.4	18.7± 4.4	0.001
ALT(U/L)	39.4± 17.8	15.0±4.2	0.001
TG(mg/dl)	142.0± 62.0	104.0± 56.0	0.02
LDL(mg/dl)	103.0 ±29.0	100.0± 28.0	0.500
HDL(mg/dl)	40.9± 7.6	47.0 ±11.0	0.01
Uric asid (mg/dl)	5.6 ±1.3	4.9 ±1.0	0.05
HOMA-IR	4.4 ± 3.0	4.1 ±2.9	0.64
Hepatostaetosis	24	26	

Body mass Index (BMI), ,Homeostatic Model of Assessment-Insulin Resistance (Homa-IR), Triglycerides (TG), High density lipoprotein (HDL), Low density lipoprotein , (LDL) , Fasting blood glucose (FBG), Aspartate Aminotransferase (AST), Alanin Aminotransferase (ALT), Calsium (Ca), Phosphour (P), Alkalen phosphatase (ALP) Parathyroid hormone (PTH)

Table 4. Patient's Demographic and Laboratory Data According to insulin Resistence (IR)

	IR (+)	IR(-)	p
Age (age)(years)	12.1± 2.09	12.2± 3.2	0,09
BMI SDS	2.7± 0.7	2.6± 0.81	0,810
Homa-IR	6.0 ± 3.1	2.3 ±0.89	0.005
Ca(mg/dl)	9.9 ±0.42	9.9± 0.39	0.409
D vit(ng/ml)	12.5± 5.7	11.4 ±5.5	0.916
PTH (pg/ml)	40.0± 14.0	53.0± 38.0	0.014
TG(mg/dl)	129.0± 71.0	102.0 ± 44.0	0.147
LDL(mg/dl)	105.0 ±30.0	97.0± 26.0	0.952
ALT(U/L)	21.0 ±13.0	23.0± 17.0	0.011
Uric asid(mg/dl)	5.2.0 ±1.0	5.0±1,3	0.670

Cutt-off values for HOMA-IR
in the prepubertal period were defined as 2.67 in boys and 2.22 in girls
in the pubertal period, 5.22 in boys and 3.82 in girls
Body mass Index (BMI), ,Homeostatic Model of Assessment-Insulin Resistance (Homa-IR), Triglycerides (TG), Low density lipoprotein , (LDL) , Aspartate Aminotransferase (AST), Alanin Aminotransferase (ALT), Calsium (Ca), Parathyroid hormone (PTH)

Table 5. hi-square for high ALT and steatosis

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	35.000a	32	.328
Likelihood Ratio	45.004	32	.063
Linear-by-Linear Association	2.646	1	.104
N of Valid Cases	35		

a. 66 cells (100,0%) have expected count less than 5. The minimum expected count is ,34.

Discussion

NAFLD is the most common chronic childhood liver disease in industrialized countries. However, fewer studies have examined the relationship between NAFLD and 25-hydroxy vitamin D levels in childhood than adulthood. This relationship needs to be investigated in order to determine the status of 25-hydroxy vitamin D in NAFLD. The main factors involved in the pathogenesis of hepatosteatosis are oxidative damage and insulin resistance. 25-hydroxy vitamin D is a powerful antioxidant. This process, caused by insulin resistance, reduces the effect of vitamin D, and low 25-hydroxy vitamin D levels contribute to insulin resistance. However, no correlation between vitamin D levels and NAFLD was observed in the study of Katz et al [6,7] .

25-hydroxy vitamin D exhibits its effects through vitamin D receptors (VDR) in the liver. The expression of VDRs decreases in hepatosteatosis and hydroxylation is also affected. The antifibrinogenic effect of $1.25(OH)_2$ vitamin D has been shown in both human and animal studies [6]. This may explain the relationship between vitamin D and hepatosteatosis. Protection against hepatosteatosis through this mechanism has been shown in vitamin D supplemented animal models. Rat studies have shown that 25-hydroxy vitamin D deficiency increases hepatosteatosis by increasing hepatic resistance and toll-like receptor activity [8]. Nutritional habits and vitamin D deficiency contribute to NAFLD through key mediators of hepatosteatosis and increasing gene expression [8,9].

The limited number of studies that have examined this relationship have produced inconsistent findings. Hourigan et al. [10] reported no significant differences in 25-hydroxy vitamin D levels between children with NAFLD proven by biopsy and children without NAFLD. Furthermore, no correlation was observed between 25-hydroxy vitamin D levels and grade of hepatosteatosis. A significant difference in 25-hydroxy vitamin D levels was reported between two sub-groups (hepatosteatosis and non-hepatosteatosis) in a study from Turkey [11]. A study from Italy reported an inverse correlation between hepatosteatosis and 25-hydroxy vitamin D levels in cases of hepatic fibrosis and hepatosteatosis proven by biopsy [12]. Adult studies have generally reported similar findings. Targher et al [13] reported a strong relationship between 25-hydroxy vitamin D levels and hepatosteatosis, necro-inflammation and fibrosis. A relationship between 25-hydroxy vitamin D levels and histopathological involvement in patients with hepatosteatosis proven by biopsy has been reported in only a few studies. In our study, we determined hepatosteatosis using non-invasive ultrasound instead of the invasive biopsy method.

A high number of patients in this study had conspicuously low 25-hydroxy vitamin D levels. Although regional differences occur in Turkey, the prevalence of 25-hydroxy vitamin D deficiency or insufficiency between the ages of 10-18 years is reported at 10-93 % in a normal population. We determined a prevalence of 95% in this study. Blood samples were taken during winter in this study, which may account for this high level. Another study from Turkey evaluated seasonal 25-hydroxy vitamin D deficiency and insufficiency in healthy schoolchildren aged [11-18]. They reported a prevalence of 93% in winter [14]. This result supports our own findings. Although we anticipated that 25-hydroxy vitamin D would affect bone metabolism, no difference was observed between the two groups in terms of serum Ca, P, ALP or PTH levels.

The main mechanisms involved in the development of NAFLD are oxidative stress injury to hepatocytes and insulin resistance. The most important risk factor for hepatosteatosis is the presence of insulin resistance [15,16]. The hepatic form of metabolic syndrome manifests as hepatosteatosis. 25-hydroxy vitamin D is known to possess antioxidant properties. Although 25-hydroxy vitamin D levels differed between the two HOMA-IR>3.16 and HOMA-IR <3.16 groups, this was not statistically significant. However, 25-hydroxy vitamin D levels were lower in the group with hepatosteatosis compared to the group without. One previous study reported that 95% of children with NAFLD had metabolic syndrome criteria and higher HOMA-IR values than the non-hepatosteatosis sub-group [16].

The parameter used to determine hepatosteatosis is usually

liver enzyme levels in obese patients. The previous upper limits for ALT levels of 50 IU/L in males and 44 IU/L in females were subsequently reduced to 25 IU/L in males and 22 IU/L in females. This shows that cases with NAFLD can have normal ALT levels and that there is no correlation between liver enzyme levels and severity of hepatosteatosis. As expected, a significant difference was determined between the hepatosteatosis and non-hepatosteatosis groups in this study. No difference was observed between 25-hydroxy vitamin D levels in cases with higher ALT levels (for females>22 IU/L and males >25 IU/L) and the normal cases.

Uric acid level is another parameter that has been proposed for use as an important biomarker. Uric acid reflects oxidative stress and insulin resistance. Recent studies have reported that high levels of uric acid are an important risk factor for NAFLD [17]. In our study, a statistically significant difference was determined between the uric acid levels of the two sub-groups ($p<0.05$). This parameter can be used to identify subjects at risk and also guide patients for imaging effectively.

One limitation of this study is that hepatosteatosis was diagnosed using ultrasound imaging. Computed tomography or magnetic resonance imaging (MRI 2D PDFF) are usually recommended for these patients. However, no advanced imaging was performed in our cases. Some studies have reported a correlation between biopsy, ultrasound imaging and liver enzymes [18]. Ultrasound imaging is unable to differentiate hepatosteatosis and hepatostatitis. This distinction can be only made by using hepatic biopsy. Although the gold standard for diagnosis of NAFLD is the hepatic biopsy, the technique is quite invasive and difficult to perform. Therefore, ultrasound imaging is often preferred in the diagnosis NAFLD rather than biopsy.

Conclusion

In conclusion, NAFLD is a multifactorial disease caused by genetic, epigenetic and environmental factors. The incidence increases proportionally with obesity. Research into the development of non-invasive methods for the diagnosis of NAFLD is currently ongoing. In our cases, no clear relationship was determined between hepatosteatosis, insulin resistance and 25-hydroxy vitamin D levels - in contrast to adults. This may be due to the samples being collected in winter. Our study is also cross-sectional in design. Long-term investigation of cases without NAFLD might reveal hepatic involvement. 25-hydroxy Vitamin D levels in cases of obesity alone may be higher in the summer. Further studies with larger patient numbers across all four seasons and under long-term observation are needed to help us better understand the relationship between NAFLD and 25-hydroxy vitamin D.

Conflict of interest
The authors declare that there are no conflicts of interest.

Competing interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Before the study, permissions were obtained from the local ethical committee.

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