THE RELATIONSHIP BETWEEN HEPATOSTEATOSIS AND VITAMIN D, CALCIUM AND MAGNESIUM

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ABSTRACT

Introduction*: Insulin resistance plays an important role in the etiology of non-alcoholic fatty liver disease. The purpose of this study is to examine the relationship between blood calcium, magnesium, and vitamin D levels, and the presence and degree of liver steatosis, which was shown to be associated with insulin resistance in some previous studies.*

Material and methods*: In this study conducted in a single-center after the approval of the ethics committee; the blood calcium, magnesium, and vitamin D levels of the patients with liver steatosis were retrospectively analyzed from the records. Those under 18 years of age, pregnant women, patients with malignancy, chronic kidney or liver disease, and diabetes were not included in the study. The data were evaluated with the NCSS program and Shapiro-Wilk, Student-t, Mann-Whitney U, Kruskal-Wallis, Dunn-Bonferroni, Pearson chi-square tests were used.*

Results*: In this study conducted with a total of 346 cases, 242 women and 104 men, the mean age of the participants was 56 years. When we divided the cases into two groups as with and without liver steatosis, the ages, genders, calcium, magnesium, and vitamin D levels of both groups were similar. When the patients with hepatosteatosis were sub-grouped regarding the degree of steatosis, these results were also similar between the subgroups (p> 0.05).*

Conclusion*: There is no relationship between the blood level of calcium, magnesium, and vitamin D and the presence and stage of fatty liver disease.*

Keywords: *Vitamin D, Calcium, Magnesium, Hepatosteatosis.*

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease. It is characterized by the deposition of the high amount of fat in the liver than it should be. Its prevalence in the world is about 20-30% and increasing day by day. NAFLD includes a wide spectrum of diseases ranging from simple fatty liver to steatohepatitis with inflammation and fibrosis. Although the gold standard in the diagnosis is liver biopsy, it is only performed in selected cases. The most common diagnostic method is ultrasound.

NAFLD can also progress to liver cirrhosis and hepatocellular cancer. Besides, NAFLD is also associated with increased cardiovascular mortality.

Insulin resistance plays an important role in the etiology of NAFLD. Vitamin D (D-vit), which plays an essential function in calcium and phosphorus metabolism and bone structure, is likewise associated with insulin resistance. Steatohepatitis is the result of inflammation and fibrosis, and Vitamin D also has anti-inflammatory and anti-fibrogenic properties. However, in previous studies about the relationship between vitamin D and NAFLD, conflicting results were obtained.

In literature research, while some studies reported that NAFLD is more common in patients with vitamin D deficiency and even improvement in liver histology and liver enzymes with vitamin D replacement, there are also some studies reporting the opposite opinion and did not find any relationship between vitamin D and NAFLD.

Similarly, while the relationship between magnesium and insulin resistance and inflammation is known, the relationship between magnesium and NAFLD has not been examined obviously before in the literature. The relationship between calcium and insulin secretion from pancreatic beta cells and the effects of insulin on target cells has been the subject of studies in previous years, but only a few studies were evaluating the relationship of calcium with NAFLD in the literature. There is no study examining the relationship between NAFLD and hypocalcemia alone in the absence of vitamin D deficiency.

Therefore, we aimed to examine the relationship between fatty liver and blood levels of Vitamin D, magnesium, and calcium in our patient population, which have been studied in a small number of studies with conflicting results.

Material and method

Ethics committee approval was obtained for this study to be conducted in a single center (Date:21.01.20, No:11). In this retrospective study, all 1-year ultrasonographic examinations requested by the internal medicine clinic for any reason between 01.01.2019 and 01.01.2020 were evaluated. Assessment of hepatosteatosis was carried out using Siemens Acuson Juniper diagnostic ultrasound system (Mountain View, CA, USA) with convex probe. The images were examined on the same monitor under the exact same lighting conditions.

Hepatosteatosis was graded according to the severity of steatosis: normal (grade 0); liver attenuation slightly less than the spleen (grade 1); more pronounced difference between liver and spleen and intrahepatic vessels not seen or at a slightly higher attenuation than the liver (grade 2); and markedly reduced liver attenuation with a sharp contrast between the liver and intrahepatic vessels (grade 3). Those who were older than 18 years of age and those who were having the blood vitamin D, calcium, magnesium, fasting blood glucose, ALT, LDL, Triglyceride (TG) values at the time of ultrasonographic examination in the system were included in the study. Pregnant women, patients with malignancy, chronic kidney or liver disease, diabetes, and individuals whose vitamin D, calcium, magnesium, fasting blood glucose, ALT, LDL, TG values were not performed at the time of ultrasonography were excluded from the study.

Statistical Analysis

NCSS (Number Cruncher Statistical System, 2007 Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, and percentage, minimum, maximum) were used while evaluating the study data. The suitability of quantitative data to normal distribution was tested by the Shapiro-Wilk test and graphical analysis. Student-t test was used for comparing normally distributed quantitative variables between two groups, and the Mann-Whitney U test was performed for comparing quantitative variables that did not show normal distribution between two groups. One-way analysis of variance and Bonferroni corrected binary evaluations were used for comparing more than two groups of quantitative variables with the normal distribution. Kruskal-Wallis test and Dunn-Bonferroni test were used to compare more than two groups of quantitative variables that did not show normal distribution. Pearson's chi-square test was used to compare qualitative data. Statistical significance was accepted as $p < 0.05$.

Results

This study was conducted with a total of 346 cases, 69.9% (n = 242) female and 30.1% (n = 104) male. The ages of the subjects included in the study ranged from 18 to 95, and the mean age was found to be 56.38 ± 17.23 years.

Although the difference regarding the ages of participants between the patients with or without hepatosteatosis was not statistically significant; the hepatosteatosis group was considerably older ($p = 0.054$; $p > 0.05$). According to the presence of hepatosteatosis, no statistically significant difference was found regarding the gender distribution, Ca, Mg and D-Vit measurements of the participants ($p > 0.05$). Fasting blood glucose, ALT, LDL, TG levels were found to be statistically significantly higher in patients with fatty liver than those without hepatosteatosis ($p < 0.05$) (Table 1).

When the cases included in the study were ultrasonographically divided into 4 groups according to the stages of fatty liver, no statistically significant

difference was found between the groups in terms of age, gender distributions, Ca, Mg, D-vit, LDL measurements (p> 0.05). A statistically significant difference was found in terms of fasting blood glucose measurements (p = 0.001; p <0.01).

		Total (n=346)	Hepatosteatosis		
			Grade 0 (n=118)	Grade1+2+3 (n=228)	P value
Age (years)	*Min-Max (Median)*	18-95 (56)	20-90 (51)	18-95 (57,5)	[c]0,054
	Mean±Sd	56,38±17,23	53,67±20,22	57,78±15,32	
Gender	**Female (n)(%)**	242 (69,9)	83 (70,3)	159 (69,7)	[b]0,908
	Male (n)(%)	104 (30,1)	35 (29,7)	69 (30,3)	
Calcium (mg/dl)	*Min-Max (Median)*	4,6-10,7 (9,51)	6,4-10,4 (9,5)	4,6-10,7 (9,6)	[c]0,123
	Mean±Sd	9,38±0,78	9,31±0,72	9,42±0,8	
Magnesium (mg/dl)	*Min-Max (Median)*	0,8-4 (1,98)	1,4-3 (1,96)	0,8-4 (1,99)	[c]0,414
	Mean±Sd	1,99±0,27	1,97±0,23	2,00±0,29	
Vitamin D (IU)	*Min-Max (Median)*	0,1-76,6 (19,8)	0,1-70,6 (19,25)	1,1-76,6 (20,13)	[c]0,276
	Mean±Sd	21,24±12,9	20,33±12,97	21,7±12,87	
FBG (mg/dl)	*Min-Max (Median)*	61-125 (95)	70-125 (91)	61-125 (96)	[c]**0,002****
	Mean±Sd	96,04±13,16	93,02±11,78	97,61±13,58	
ALT (IU/ml)	*Min-Max (Median)*	3-102 (17)	4-102 (15)	3-95 (18)	[c]**0,009****
	Mean±Sd	20,87±14,56	18,83±14,4	21,93±14,56	
LDL (mg/dl)	*Min-Max (Median)*	24-243 (125)	57-242 (113,5)	24-243 (131,5)	[c]**0,036***
	Mean±Sd	127±44,5	120,03±41	130,61±45,87	
Triglyceride (mg/dl)	*Min-Max (Median)*	38-395 (115,5)	38-377 (97)	42-395 (137,5)	[c]**0,001****
	Mean±Sd	133,77±68,01	108,45±55,27	146,88±70,35	

Table 1: Demographic and biochemical data in the presence and absence of hepatosteatosis.
*[a]Student-t Test, [b]Pearson Chi-Square Test, [c]Mann Whitney U Test, *p<0,05, **p<0,01*

According to the results of the paired comparisons made to determine the difference; the blood glucose level of patients without hepatosteatosis was found to be significantly lower than those with grade 2 or 3 hepatosteatosis (p = 0.001; p = 0.029, respectively; p <0.05). Likewise, blood glucose levels of patients with grade 1 hepatosteatosis were found to be significantly lower than those with grade 3 (p = 0.016; p <0.05). Again, a statistically significant difference was found between ALT measurements of the cases (p = 0.001; p <0.01).

According to the results of paired comparison made to determine the difference; ALT values of cases without hepatosteatosis were found to be significantly lower than those with grade 2 or 3 (p = 0.014; p = 0.005, respectively; p <0.05). Likewise, ALT values of grade 1 cases were found to be significantly lower than those with grade 3 hepatosteatosis (p = 0.030; p <0.05). When the groups were compared in terms of triglyceride measurements, a statistically significant difference was found (p = 0.001; p <0.01). According to the results of paired comparisons made to determine the difference; triglyceride levels of the cases without hepatosteatosis were found to be significantly lower than the cases with grade 1, 2 and 3 hepatosteatosis (p = 0.011; p = 0.001; p = 0.001, respectively; p <0.01). Likewise, the triglyceride levels of grade 1 cases were found to be significantly lower than those with grade 2 hepatosteatosis (p = 0.001; p <0.01) (Table 2).

Hepatosteatosis		Grade 0 (n=118)	Grade 1 (n=139)	Grade 2 (n=70)	Grade 3 (n=19)	P value
Age (years)	*Min-Max (Median)*	20-90 (51)	18-95 (58)	22-86 (56)	35-80 (55)	[d]0,213
	Mean±Sd	53,67±20,22	58,48±16,12	56,24±14,06	58,32±14,06	
Gender	**Female**	83 (70,3)	92 (66,2)	52 (74,3)	15 (78,9)	[b]0,515
	Male	35 (29,7)	47 (33,8)	18 (25,7)	4 (21,1)	
Calcium (mg/dl)	*Min-Max (Median)*	6,4-10,4 (9,5)	4,6-10,7 (9,5)	7,2-10,7 (9,65)	7,9-10,5 (9,5)	[d]0,163
	Mean±Sd	9,31±0,72	9,36±0,87	9,55±0,68	9,41±0,65	
Magnesium (mg/dl)	*Min-Max (Median)*	1,4-3 (1,96)	0,8-4 (2,01)	1,3-2,6 (1,97)	1,5-2,6 (1,96)	[d]0,110
	Mean±Sd	1,97±0,23	2,03±0,32	1,95±0,22	1,92±0,28	
2Vitamin D (IU)	*Min-Max (Median)*	0,1-70,6 (19,25)	1,1-66,4 (20,17)	1,6-67,1 (20,29)	5,3-76,6 (18,23)	[d]0,747
	Mean±Sd	20,33±12,97	21,55±13,06	21,32±10,96	24,26±17,73	
FBG (mg/dl)	*Min-Max (Median)*	70-125 (91)	61-125 (95)	65-124 (99,5)	70-117 (104)	[d]**0,001****
	Mean±Sd	93,02±11,78	95,31±13,62	100,99±12,75	102±13,26	
ALT (IU/ml)	*Min-Max (Median)*	4-102 (15)	3-67 (16)	4-95 (21)	8-68 (25)	[d]**0,001****
	Mean±Sd	18,83±14,4	19,73±12,14	24,59±17,84	28,16±14,82	
LDL (mg/dl)	*Min-Max (Median)*	57-242 (113,5)	24-243 (130)	37-232 (131,5)	46-188 (142)	[d]0,164
	Mean±Sd	120,03±41	129,14±47,99	132,93±42,51	132,74±43,78	
Triglyceride (mg/dl)	*Min-Max (Median)*	38-377 (97)	42-386 (115)	51-395 (162,5)	62-321 (152)	[d]**0,001****
	Mean±Sd	108,45±55,27	131,79±63,6	168,87±72,54	176,26±81,71	

Table 2: Demographic and biochemical data according to the ultrasonographic staging of hepatosteatosis.
*[a]Student-t Test, [d]Oneway ANOVA, [e]Kruskal Wallis Test, **p<0,01*

Discussion

The relationship between NAFLD and insulin resistance is well known. However, the relationship between the blood levels of Ca, Mg, and vitamin D, which are also known to be associated with insulin resistance, with the presence and degree of hepatosteatosis is still not fully elucidated.

Recently, Liu et al. evaluated fifteen studies and found that there was a negative relationship between blood vitamin D levels and NAFLD, and NAFLD improved with vitamin D supplementation[1]. Similarly, in many other studies, the relationship between vitamin D and NAFLD has been shown[1-8]. However, in many studies in the literature, a relationship between vitamin D and NAFLD was not determined[9-16].

Therefore, the association of vitamin D and NAFLD is still doubtful despite many studies. In our study, no relationship was found between the vitamin D level and the presence or degree of fatty liver.

Magnesium, which is the second most intracellular cation and acts as a cofactor, has anti-inflammatory and insulin resistance reducing properties[17]. While magnesium supplementation has been shown to have a lowering effect on HOMA-IR in a meta-analysis, it has been found that magnesium supplementation lowers fasting glucose in diabetic patients in some other studies[18-20]. One of the few studies in the literature examining the relationship between magnesium and NAFLD is the study of Eshraghian et al. As a result of this study supported by liver biopsy, serum magnesium levels were found to be independently and statistically significantly associated with both hepatosteatosis and steatohepatitis[21]. However, as a result of our study, no relationship was found with Mg and the presence or degree of fatty liver disease.

In one of the few studies showing the relationship between calcium and diabetes, Pittas et al. found that while low vitamin D and calcium levels negatively affect glycemia, their combined consumption may regulate glucose metabolism[22]. Again, in another study, it was reported that combined therapy would improve NAFLD[23]. There is also a study in the literature that found that the intake of vitamin D together with calcium, not alone, improves liver functions and decreases the degree of liver fat in NAFLD[24]. However, the number of these studies is quite low, and in a previous study regarding the association of calcium and NAFLD, no relationship was found between calcium intake and hepatosteatosis. When the sub-breakdowns of the same study were done, a statistically significant relationship was found between calcium intake and hepatosteatosis only in women[25]. However, as a result of our study, no relationship was found between Ca and the presence or degree of fatty liver disease.

The limitations of this study are that it was conducted in a single center and the diagnosis and staging of fatty liver disease was not performed by biopsy.

Based on the results of our study, we can say that the presence and stage of NAFLD do not correlate with the blood Ca, Mg, and vitamin D levels.

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