**Name of Journal:** *World Journal of Gastrointestinal Surgery*

**Manuscript NO:** 53290

**Manuscript Type:** ORIGINAL ARTICLE

***Retrospective Study***

**Role of micronutrients in staging of nonalcoholic fatty liver disease: A retrospective cross-sectional study**

Bertol FS *et al*. Staging of NAFLD

Franciele Sabadin Bertol, Bruna Araujo, Brunno Brochado Jorge, Natalino Rinaldi, Luiz Alberto De Carli, Cristiane Valle Tovo

**Franciele Sabadin Bertol, Bruna Araujo, Brunno Brochado Jorge, Natalino Rinaldi, Luiz Alberto De Carli, Cristiane Valle Tovo,** Graduate Program of Medicine, Hepatology, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS 90430080, Brazil

**Author contributions:** Tovo CV conceptualized the manuscript; Bertol FS, Araujo B and Jorge BB collected the data; Bertol FS and Tovo CV wrote the manuscript; All authors helped to perform the research, performed the final review with significant contributions, and approved the final version of the manuscript.

**Corresponding author: Cristiane Valle Tovo,** **MD, PhD, Adjunct Professor,** Graduate Program of Medicine, Hepatology, Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite 245, Porto Alegre, RS 90430080, Brazil. [cristianev@ufcspa.edu.br](mailto:cristianev@ufcspa.edu.br)

**Received:** December 15, 2019

**Revised:** April 10, 2020

**Accepted:** May 5, 2020

**Published online:**

**Abstract**

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) presents high incidence throughout the world and has been progressively increasing in prevalence. This disease has a heterogeneous natural history, including simple steatosis, nonalcoholic steatohepatitis (NASH), and cirrhosis. The factors that determine its evolution to more severe forms of the disease are still poorly understood, and micronutrients with antioxidant potential may be involved in the pathophysiology of the disease.

AIM

To evaluate the relationship between serum levels of micronutrients and the severity of NAFLD.

METHODS

A retrospective, observational and cross-sectional study was conducted. This study included all patients undergoing bariatric surgery who experienced liver biopsy during the procedure, and had serum levels of micronutrients (vitamin D, vitamin B12, zinc, iron, and magnesium), which was assessed in a preoperative evaluation conducted at a reference center in southern Brazil.

RESULTS

A total of 614 patients were analyzed, of which 93% had steatosis, 70.7% had NASH, and 49.3% had some degree of fibrosis. Serum levels of vitamin D were negatively correlated with the severity of steatosis and NASH, and serum levels of vitamin B12 were positively correlated with the severity of steatosis and fibrosis. The other micronutrients showed no association with NAFLD staging.

CONCLUSION

Serum levels of vitamin D are inversely related to the severity of steatosis and NASH, and serum levels of vitamin B12 are higher in more advanced stages of simple steatosis and liver fibrosis. Serum levels of zinc, iron, and magnesium were not associated with NAFLD severity.

**Key words:** Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Liver cirrhosis; Micronutrients; Vitamin D; Vitamin B12; Zinc; Iron; Magnesium

Bertol FS, Araujo B, Jorge BB, Rinaldi N, De Carli LA, Tovo CV. Role of micronutrients in staging of nonalcoholic fatty liver disease: A retrospective cross-sectional study. *World J Gastrointest Surg* 2020; In press

**Core tip:** Nonalcoholic fatty liver disease presents with a high incidence throughout the world, and micronutrients may be involved in the pathophysiology of the disease. This study evaluated the relationship between serum levels of micronutrients and the severity of nonalcoholic fatty liver disease. Six hundred and fourteen patients undergoing bariatric surgery that had serum levels of micronutrients (vitamin D, vitamin B12, zinc, iron, and magnesium) as assessed in a preoperative period were included. Vitamin D levels were negatively correlated with the severity of steatosis and nonalcoholic steatohepatitis, and vitamin B12 levels were positively correlated with the severity of steatosis and fibrosis. In conclusion, vitamin D and vitamin B12 are related to the severity of steatosis, nonalcoholic steatohepatitis, and liver fibrosis.

**INTRODUCTION**

Nonalcoholic fatty liver disease (NAFLD) presents with a high incidence throughout the world, affecting about 20%-25% of the population[1-3]. Its prevalence is increasing due to changes in lifestyle and the obesity epidemic; besides, it is believed that by 2020, NAFLD will be the leading cause of chronic liver disease, which will also increase public expenditures worldwide[3,4].

This disease is considered to be a hepatic manifestation of the metabolic syndrome, causing an increase in overall mortality that is mainly related to cardiovascular diseases[5-9]. Obesity is the main risk factor for the disease[10].

The pathogenesis of NAFLD is heterogeneous and not yet fully understood, encompassing simple steatosis, nonalcoholic steatohepatitis (NASH), and cirrhosis as consequences. It is of crucial importance to identify the factors that lead to disease progression to find possible therapeutic targets.

Just as excess of macronutrients contributes to tissue damage and alteration of energy homeostasis in NAFLD patients, they may also contribute to disease progression, disrupting lipid homeostasis and antioxidant mechanisms[11].

NAFLD also promotes mineral deficiency[12,13], and thus changes in the metabolism of vitamin D, vitamin B12, zinc, iron, and magnesium may be related to histological and/or clinical events in NAFLD patients. However, studies that evaluate the relationship between micronutrients and NAFLD are scarce, and present controversial results[11,13-17].

The present study aims to analyze the correlation between NAFLD staging and the serum levels of micronutrients (vitamin D, vitamin B12, zinc, iron, and magnesium) in obese patients.

**MATERIALS AND METHODS**

A retrospective cross-sectional study was developed for patients treated between 2002 and 2016 at the Center for Obesity Treatment of the Hospital Santa Casa de Misericórdia de Porto Alegre, RS, Brazil, a tertiary referral hospital in southern Brazil.

The inclusion criteria were patients who underwent liver biopsy during bariatric surgery and who had measured serum levels of at least one of the micronutrients studied (vitamin D, vitamin B12, zinc, iron, and magnesium) during outpatient evaluation prior to the surgical procedure.

The exclusion criteria were patients with viral hepatitis B or C, HIV, other causes of chronic liver disease, significant alcohol consumption (> 21 doses per week for men and > 14 doses per week), use of potentially steatogenic drugs, or secondary causes of NAFLD[18].

Liver biopsies were analyzed by a professional with experience in liver pathology, according to the criteria of Kleiner *et al*[19].

Clinical and laboratory data were collected during a previous evaluation performed up to 1 year before bariatric surgery. The variables of age, sex, body mass index (BMI), presence of comorbidities [diabetes mellitus (DM), DM complications, systemic arterial hypertension (SAH), sleep apnea, smoking, and dyslipidemia], vitamin D, vitamin B12, zinc, iron, and magnesium were analyzed.

Patients were stratified according to the World Health Organization classification for obesity (Class I: BMI between 30 and 35 kg/m2; Class II: BMI between 35 and 40 kg/m2; Class III: BMI > 40 kg/m2)[20].

All analyses were tabulated and processed using the Statistical Package for the Social Sciences program (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc), and the significance level was set at 5%.

Means and standard deviations were described for variables with normal distribution, and medians were described for variables that did not present normal distributions, which was verified by the Kolmogorov-Smirnov test.

The Kruskal-Wallis test was used to correlate the degrees of hepatic steatosis with serum levels of the different micronutrients. The Mann-Whitney test was used to compare the serum levels of micronutrients between patients with mild/absent steatosis, NASH, and fibrosis, as well as those with advanced steatosis, NASH, and fibrosis.

**RESULTS**

Of the 1752 patients who had performed bariatric surgery during the study period, 1138 were excluded because they did not undergo liver biopsy. This study thus included 614 patients.

The mean patient age was 37.2 ± 9.7 years, 79% were female, and 83.1% had some comorbidity (17.2% DM, 41.7% SAH, 14% sleep apnea, 77% smoking, and 40.9% dyslipidemia).

When liver histopathology was evaluated, 93% of patients had simple steatosis, 70.7% had NASH (grade 1 in 48.3%; grade 2 in 21.4%; grade 3 in 1%), and 49.3 % had fibrosis (grade 1 in 37.2%; grade 2 in 5.8%; grade 3 in 6.3%; grade 4 in 0.5%).

According to the preoperative BMI, most patients (77.1%) presented class III obesity. The medians of micronutrient levels (vitamin D, vitamin B12, zinc, iron, and magnesium) were within the normal range. These results are described in Table 1.

Vitamin D values were higher in patients with grade 1 steatosis than in those with grades 2 or 3; and vitamin B12 levels were higher in patients with grade 3 steatosis compared to those with grades 1 or 2 steatosis. There was no statistically significant difference in mean serum levels of micronutrients according to the different grades of NASH or fibrosis (Table 2).

Vitamin D was significantly higher in patients with absent/mild steatosis compared with patients with advanced steatosis; vitamin B12 was higher in patients with advanced fibrosis compared to patients with absent/mild fibrosis (Table 3).

There was a negative correlation between vitamin D levels and the grade of steatosis and NASH, and a positive correlation between vitamin B12 levels and the grade of steatosis and fibrosis (Table 4).

**DISCUSSION**

It has recently been suggested that some micronutrients would have antioxidant potential and could reduce the accumulation of reactive oxygen species, consequently delaying or preventing the evolution of NAFLD[11,21].

The present study evaluated the serum levels of vitamin D, vitamin B12, zinc, iron, and magnesium, and their relationship with NAFLD staging. Significantly higher serum levels of vitamin D were observed in patients with absent/mild steatosis compared to patients with advanced steatosis; and significantly higher serum levels of vitamin B12 were observed in patients with advanced fibrosis compared to patients with absent/mild fibrosis. There was also a negative correlation between vitamin D and the grade of steatosis and NASH, and a positive correlation between vitamin B12 levels and the grade of steatosis and fibrosis. No correlation was found between the staging of NAFLD and serum levels of zinc, iron, and magnesium.

In the literature, studies evaluating the relationship between micronutrients and NAFLD are scarce and controversial.

Vitamin D may affect the development and course of liver diseases, mainly due to its immunomodulatory role[22]. It may also inhibit the formation of human type I collagen in stellate cells, suggesting a relationship between vitamin D deficiency and the progression of liver fibrosis[23]. In addition, low levels of vitamin D have been associated with the severity of steatosis, NASH, and fibrosis in NAFLD[24,25]. Vitamin D deficiency has also been associated with increased insulin resistance and exacerbation of hepatic steatosis in obese rats[26]; besides, studies on this subject are scarce and inconclusive. The present study found a higher vitamin D level in patients with absent/mild steatosis compared to patients with advanced steatosis, which is in agreement with most published studies[23-26].

The association between vitamin B12 and NAFLD remains controversial. Some authors report similar[15,27], higher[28,29], or lower[30] serum levels in NAFLD patients compared to control patients. Vitamin B12 reduction could be associated with insulin resistance and endothelial dysfunction[31]. The present study presented a positive correlation between serum levels of vitamin B12 and staging of NAFLD, and patients with advanced fibrosis had higher serum levels of this vitamin.

Zinc has been implicated in the pathogenesis of liver diseases of various etiologies[17,32]. Some studies obtained a good correlation between zinc deficiency and hepatic steatosis in experimental models of hepatic steatosis[12,33]. However, there are no studies with human subjects that show a correlation between serum levels of zinc in NAFLD and disease severity. In the presenting study, the serum levels of zinc showed no statistically significant difference between the grades of steatosis, NASH, or fibrosis.

Iron has also been implicated in the pathogenesis of NAFLD, but its role is not yet fully elucidated. The relationship between serum levels of ferritin and staging of NAFLD has been reported; however, ferritin is an inaccurate measure of iron storages[34,35]. Ferritin levels 1.5x above the normal range have been independently associated with the grade of fibrosis in patients with NASH[16,36]. However, some studies did not corroborate this association[37-40]. The present study evaluated the serum levels of iron in patients with NAFLD, which did not correlate with the severity of NASH or fibrosis.

Serum levels of magnesium have been independently associated with the presence of steatosis and NASH compared to healthy individuals[41]. Inverse correlations have also been observed between the serum levels of magnesium and glycemic indices in patients with insulin resistance[42]. In the present study, however, no statistically significant correlation was found between the serum levels of magnesium and staging of NAFLD.

Although this study found a relationship between the severity of NAFLD and serum levels of vitamin D and vitamin B12, its clinical relevance is questionable, as these are weak correlations. However, possible limitations include the fact that this was a retrospective study, due to the large number of patients analyzed. The importance of the observed data should therefore be considered.

In conclusion, the serum levels of vitamin D are inversely related to steatosis and the severity of NASH, and the serum levels of vitamin B12 are higher in more advanced stages of steatosis and liver fibrosis. Serum levels of zinc, iron, and magnesium are not associated with the severity of NAFLD.

**ARTICLE HIGHLIGHTS**

***Research background***

It has been suggested that some micronutrients would have antioxidant potential and could reduce the accumulation of reactive oxygen species, consequently delaying or preventing the evolution of nonalcoholic fatty liver disease (NAFLD).

***Research motivation***

Identify potential new therapeutic strategies for NAFLD.

***Research objectives***

Evaluate the relationship between serum levels of micronutrients and the severity of NAFLD.

***Research methods***

A retrospective, observational, and cross-sectional study was conducted. This study included patients undergoing bariatric surgery at a reference center in southern Brazil. These patients underwent liver biopsy during the procedure and had serum levels of micronutrients assessed in a preoperative evaluation.

***Research results***

Serum levels of vitamin D were negatively correlated with the severity of steatosis and nonalcoholic steatohepatitis, and serum levels of vitamin B12 were positively correlated with the severity of steatosis and fibrosis.

***Research conclusions***

Serum levels of vitamin D are inversely related to the severity of steatosis and nonalcoholic steatohepatitis, and serum levels of vitamin B12 are higher in more advanced stages of simple steatosis and liver fibrosis.

***Research perspectives***

Further studies should be done to assess the relationship of micronutrients and NAFLD.

**REFERENCES**

1 **Angulo P**, Lindor KD. Non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2002; **17** Suppl: S186-S190 [PMID: 12000605 DOI: 10.1046/j.1440-1746.17s1.10.x]

2 **Younossi ZM**, Diehl AM, Ong JP. Nonalcoholic fatty liver disease: an agenda for clinical research. *Hepatology* 2002; **35**: 746-752 [PMID: 11915019 DOI: 10.1053/jhep.2002.32483]

3 **Asrani SK**, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol* 2019; **70**: 151-171 [PMID: 30266282 DOI: 10.1016/j.hep.2018.09.014]

4 **Ofosu A**, Ramai D, Reddy M. Non-alcoholic fatty liver disease: controlling an emerging epidemic, challenges, and future directions. *Ann Gastroenterol* 2018; **31**: 288-295 [PMID: 29720854 DOI: 10.20524/aog.2018.0240]

5 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

6 **Musso G**, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, Hultcrantz R, Hagström H, Yoon SK, Charatcharoenwitthaya P, George J, Barrera F, Hafliðadóttir S, Björnsson ES, Armstrong MJ, Hopkins LJ, Gao X, Francque S, Verrijken A, Yilmaz Y, Lindor KD, Charlton M, Haring R, Lerch MM, Rettig R, Völzke H, Ryu S, Li G, Wong LL, Machado M, Cortez-Pinto H, Yasui K, Cassader M. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. *PLoS Med* 2014; **11**: e1001680 [PMID: 25050550 DOI: 10.1371/journal.pmed.1001680]

7 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]

8 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047 DOI: 10.2337/diabetes.50.8.1844]

9 **Popov VB**, Lim JK. Treatment of Nonalcoholic Fatty Liver Disease: The Role of Medical, Surgical, and Endoscopic Weight Loss. *J Clin Transl Hepatol* 2015; **3**: 230-238 [PMID: 26623270 DOI: 10.14218/JCTH.2015.00019]

10 **Wu R**, Ortiz J, Dallal R. Is bariatric surgery safe in cirrhotics? *Hepat Mon* 2013; **13**: e8536 [PMID: 23610589 DOI: 10.5812/hepatmon.8536]

11 **Pickett-Blakely O**, Young K, Carr RM. Micronutrients in Nonalcoholic Fatty Liver Disease Pathogenesis. *Cell Mol Gastroenterol Hepatol* 2018; **6**: 451-462 [PMID: 30294653 DOI: 10.1016/j.jcmgh.2018.07.004]

12 **Shidfar F**, Faghihi A, Amiri HL, Mousavi SN. Regression of Nonalcoholic Fatty Liver Disease with Zinc and Selenium Co-supplementation after Disease Progression in Rats. *Iran J Med Sci* 2018; **43**: 26-31 [PMID: 29398749]

13 **Sánchez A**, Rojas P, Basfi-Fer K, Carrasco F, Inostroza J, Codoceo J, Valencia A, Papapietro K, Csendes A, Ruz M. Micronutrient Deficiencies in Morbidly Obese Women Prior to Bariatric Surgery. *Obes Surg* 2016; **26**: 361-368 [PMID: 26108638 DOI: 10.1007/s11695-015-1773-9]

14 **Kwok RM**, Torres DM, Harrison SA. Vitamin D and nonalcoholic fatty liver disease (NAFLD): is it more than just an association? *Hepatology* 2013; **58**: 1166-1174 [PMID: 23504808 DOI: 10.1002/hep.26390]

15 **Polyzos SA**, Kountouras J, Patsiaoura K, Katsiki E, Zafeiriadou E, Zavos C, Deretzi G, Tsiaousi E, Slavakis A. Serum vitamin B12 and folate levels in patients with non-alcoholic fatty liver disease. *Int J Food Sci Nutr* 2012; **63**: 659-666 [PMID: 22229957 DOI: 10.3109/09637486.2011.649249]

16 **Kowdley KV**, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, Sanyal AJ, Nelson JE; NASH Clinical Research Network. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 77-85 [PMID: 21953442 DOI: 10.1002/hep.24706]

17 **Himoto T**, Nomura T, Tani J, Miyoshi H, Morishita A, Yoneyama H, Haba R, Masugata H, Masaki T. Exacerbation of insulin resistance and hepatic steatosis deriving from zinc deficiency in patients with HCV-related chronic liver disease. *Biol Trace Elem Res* 2015; **163**: 81-88 [PMID: 25413880 DOI: 10.1007/s12011-014-0177-3]

18 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ; American Gastroenterological Association; American Association for the Study of Liver Diseases; American College of Gastroenterologyh. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

19 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

20 Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000; **894**: i-xii, 1-253 [PMID: 11234459]

21 **Chen G**, Ni Y, Nagata N, Xu L, Ota T. Micronutrient Antioxidants and Nonalcoholic Fatty Liver Disease. *Int J Mol Sci* 2016; **17**: 1379 [PMID: 27563875 DOI: 10.3390/ijms17091379]

22 **Zúñiga S**, Firrincieli D, Housset C, Chignard N. Vitamin D and the vitamin D receptor in liver pathophysiology. *Clin Res Hepatol Gastroenterol* 2011; **35**: 295-302 [PMID: 21440524 DOI: 10.1016/j.clinre.2011.02.003]

23 **Potter JJ**, Liu X, Koteish A, Mezey E. 1,25-dihydroxyvitamin D3 and its nuclear receptor repress human α1 (I) collagen expression and type I collagen formation. *Liver Int* 2013; **33**: 677-686 [PMID: 23413886 DOI: 10.1111/liv.12122]

24 **Targher G**, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, Arcaro G. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2007; **17**: 517-524 [PMID: 16928437 DOI: 10.1016/j.numecd.2006.04.002]

25 **Skaaby T**, Husemoen LL, Borglykke A, Jørgensen T, Thuesen BH, Pisinger C, Schmidt LE, Linneberg A. Vitamin D status, liver enzymes, and incident liver disease and mortality: a general population study. *Endocrine* 2014; **47**: 213-220 [PMID: 24272594 DOI: 10.1007/s12020-013-0107-8]

26 **Roth CL**, Elfers CT, Figlewicz DP, Melhorn SJ, Morton GJ, Hoofnagle A, Yeh MM, Nelson JE, Kowdley KV. Vitamin D deficiency in obese rats exacerbates nonalcoholic fatty liver disease and increases hepatic resistin and Toll-like receptor activation. *Hepatology* 2012; **55**: 1103-1111 [PMID: 21994008 DOI: 10.1002/hep.24737]

27 **Hirsch S**, Poniachick J, Avendaño M, Csendes A, Burdiles P, Smok G, Diaz JC, de la Maza MP. Serum folate and homocysteine levels in obese females with non-alcoholic fatty liver. *Nutrition* 2005; **21**: 137-141 [PMID: 15723740 DOI: 10.1016/j.nut.2004.03.022]

28 **Gulsen M**, Yesilova Z, Bagci S, Uygun A, Ozcan A, Ercin CN, Erdil A, Sanisoglu SY, Cakir E, Ates Y, Erbil MK, Karaeren N, Dagalp K. Elevated plasma homocysteine concentrations as a predictor of steatohepatitis in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2005; **20**: 1448-1455 [PMID: 16105135 DOI: 10.1111/j.1440-1746.2005.03891.x]

29 **Koplay M**, Gulcan E, Ozkan F. Association between serum vitamin B12 levels and the degree of steatosis in patients with nonalcoholic fatty liver disease. *J Investig Med* 2011; **59**: 1137-1140 [PMID: 21804402 DOI: 10.2310/JIM.0b013e31822a29f5]

30 **Sazci A**, Akpinar G, Aygun C, Ergul E, Senturk O, Hulagu S. Association of apolipoprotein E polymorphisms in patients with non-alcoholic steatohepatitis. *Dig Dis Sci* 2008; **53**: 3218-3224 [PMID: 18465245 DOI: 10.1007/s10620-008-0271-5]

31 **Setola E**, Monti LD, Galluccio E, Palloshi A, Fragasso G, Paroni R, Magni F, Sandoli EP, Lucotti P, Costa S, Fermo I, Galli-Kienle M, Origgi A, Margonato A, Piatti P. Insulin resistance and endothelial function are improved after folate and vitamin B12 therapy in patients with metabolic syndrome: relationship between homocysteine levels and hyperinsulinemia. *Eur J Endocrinol* 2004; **151**: 483-489 [PMID: 15476449 DOI: 10.1530/eje.0.1510483]

32 **Grüngreiff K**. Zinc in liver disease. *J Trace Elem Exp Med* 2002; **15**: 67-78 [DOI: 10.1002/jtra.10002]

33 **Mikhail TH**, Nicola WG, Ibrahim KH, Salama SH, Emam M. Abnormal zinc and copper metabolism in hepatic steatosis. *Boll Chim Farm* 1996; **135**: 591-597 [PMID: 9048448]

34 **Britton LJ**, Subramaniam VN, Crawford DH. Iron and non-alcoholic fatty liver disease. *World J Gastroenterol* 2016; **22**: 8112-8122 [PMID: 27688653 DOI: 10.3748/wjg.v22.i36.8112]

35 **Bugianesi E**, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179-187 [PMID: 14752836 DOI: 10.1002/hep.20023]

36 **George DK**, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, Jazwinska EC, Powell LW. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1998; **114**: 311-318 [PMID: 9453491 DOI: 10.1016/s0016-5085(98)70482-2]

37 **Valenti L**, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, Canavesi E, Lattuada E, Roviaro G, Marchesini G, Fargion S. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010; **138**: 905-912 [PMID: 19931264 DOI: 10.1053/j.gastro.2009.11.013]

38 **Chandok N**, Minuk G, Wengiel M, Uhanova J. Serum ferritin levels do not predict the stage of underlying non-alcoholic fatty liver disease. *J Gastrointestin Liver Dis* 2012; **21**: 53-58 [PMID: 22457860]

39 **Fargion S**, Mattioli M, Fracanzani AL, Sampietro M, Tavazzi D, Fociani P, Taioli E, Valenti L, Fiorelli G. Hyperferritinemia, iron overload, and multiple metabolic alterations identify patients at risk for nonalcoholic steatohepatitis. *Am J Gastroenterol* 2001; **96**: 2448-2455 [PMID: 11513189 DOI: 10.1111/j.1572-0241.2001.04052.x]

40 **Bonkovsky HL**, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht RW, Banner BF. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 421-429 [PMID: 10488699 DOI: 10.1016/s0168-8278(99)800032-4]

41 **Eshraghian A**, Nikeghbalian S, Geramizadeh B, Malek-Hosseini SA. Serum magnesium concentration is independently associated with non-alcoholic fatty liver and non-alcoholic steatohepatitis. *United European Gastroenterol J* 2018; **6**: 97-103 [PMID: 29435319 DOI: 10.1177/2050640617707863]

42 **Yadav C**, Manjrekar PA, Agarwal A, Ahmad A, Hegde A, Srikantiah RM. Association of Serum Selenium, Zinc and Magnesium Levels with Glycaemic Indices and Insulin Resistance in Pre-diabetes: a Cross-Sectional Study from South India. *Biol Trace Elem Res* 2017; **175**: 65-71 [PMID: 27272715 DOI: 10.1007/s12011-016-0766-4]

**Footnotes**

**Institutional review board statement:** This study agrees with resolution 466 of 2012, which governs the conduct of human subjects research, and was approved by the Research Ethics Committee of the institution concerned (opinion number 982.654).

**Informed consent statement:** Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment.

**Conflict-of-interest statement:** The authors declare no conflicts of interest.

**Data sharing statement:** No additional data are available. Data used and/or analyzed during this study are available upon request to the author.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Peer-review started:** December 15, 2019

**First decision:** April 2, 2020

**Article in press:**

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Brazil

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C, C, C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Ahmed M, Jin C, Treeprasertsuk S **S-Editor:** Ma RY **L-Editor:** Filipodia **E-Editor:**

**Table 1 Sample characteristics**

|  |  |
| --- | --- |
| **Characteristics** | **Value** |
| Age mean and standard deviation | 37 ± 9.68 |
| Female sex, *n* (%) | 455 (79.0) |
| BMI, mean, and standard deviation | 42.8 ± 5.57 |
| BMI classification, *n* (%) |  |
| Obesity Class I | 04 (0.7) |
| Obesity Class II | 121 (22.2) |
| Obesity Class III | 420 (77.1) |
| Comorbidities, *n* (%) | 446 (83.1) |
| DM | 95 (17.2) |
| DM complications | 11 (8.5) |
| SAH | 234 (41.7) |
| Sleep apnea | 75 (14) |
| Smoking | 41 (7.7) |
| Dyslipidemia | 210 (40.9) |
| Micronutrients, median (min-max) |  |
| Vitamin D, pg/mL | 20 (4.2-52.2) |
| Vitamin B12, pg/mL | 407 (36-2000) |
| Zinc, mg/L | 6.6 (4-19.4) |
| Iron, mcg/dL | 79 (8-408) |
| Magnesium, mg/dL | 2.1 (1.3-2.9) |
| Steatosis, *n* (%) | 604 (100.0) |
| 0 | 42 (7.0) |
| 1 | 221 (36.6) |
| 2 | 182 (30.1) |
| 3 | 159 (26.3) |
| NASH, *n* (%) | 584 (100.0) |
| 0 | 171 (29.3) |
| 1 | 282 (48.3) |
| 2 | 125 (21.4) |
| 3 | 06 (1.0) |
| Fibrosis, *n* (%) | 602 (100.0) |
| 0 | 302 (50.2) |
| 1 | 224 (37.2) |
| 2 | 35 (5.8) |
| 3 | 38 (6.3) |
| 4 | 03 (0.5) |

*n* = 614,sample size. BMI: Body mass index; DM: Diabetes mellitus; NASH: Nonalcoholic steatohepatitis; SAH: Systemic arterial hypertension.

**Table 2 Mean serum levels of micronutrients according to liver histopathology staging**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Grade** | **Vitamin D, pg/mL** | **Vitamin B12, pg/mL** | **Zinc, mg/L** | **Iron, mcg/dL** | **Magnesium, mg/dL** |
| Steatosis | 0 | 21 | 433 | 6.7 | 82 | 2.2 |
|  | 1 | 21 | 390 | 6.6 | 78 | 2.0 |
|  | 2 | 19 | 400 | 6.6 | 77 | 2.1 |
|  | 3 | 19 | 444 | 6.6 | 80 | 2.1 |
|  | *P* value | 0.047 | 0.040 | 0.681 | 0.959 | 0.166 |
| NASH | 0 | 21 | 416 | 6.7 | 76 | 2.1 |
|  | 1 | 20 | 395 | 6.6 | 79 | 2.0 |
|  | 2 | 18 | 404 | 6.5 | 79 | 2.0 |
|  | 3 | - | 304 | - | 80 | - |
|  | *P* value | 0.198 | 0.553 | 0.950 | 0.521 | 0.536 |
| Fibrosis | 0 | 20 | 401 | 6.8 | 79 | 2.1 |
|  | 1 | 21 | 400 | 6.6 | 75 | 2.1 |
|  | 2 | 19 | 447 | 6.8 | 82 | 2.0 |
|  | 3 | 17 | 444 | 6.8 | 83 | 2.0 |
|  | 4 | - | - | - | - | - |
|  | *P* value | 0.732 | 0.110 | 0.877 | 0.468 | 0.316 |

Kruskal-Wallis test. Insufficient numbers for comparison are represented with “-“. NASH: Nonalcoholic steatohepatitis.

**Table 3 Serum levels of micronutrients and liver histopathological analysis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Vitamin D, pg/mL** | **Vitamin B12, pg/mL** | **Zinc, mg/L** | **Iron, mcg/dL** | **Magnesium, mg/dL** |
| Steatosis | Absent/mild | 21 | 394 | 6.6 | 78 | 2.0 |
|  | Advanced | 19 | 418 | 6.6 | 79 | 2.1 |
|  | *P* value | 0.008 | 0.134 | 0.618 | 0.814 | 0.961 |
| NASH | Absent/mild | 21 | 402 | 6.6 | 77 | 2.1 |
|  | Advanced | 18 | 403 | 6.5 | 79 | 2.0 |
|  | *P* value | 0.135 | 0.960 | 0.900 | 0.726 | 0.562 |
| Fibrosis | Absent/mild | 20 | 402 | 6.6 | 78 | 2.1 |
|  | Advanced | 17 | 477 | 6.8 | 83 | 2.0 |
|  | *P* value | 0.182 | 0.030 | 0.858 | 0.457 | 0.149 |

Mann-Whitney test. Absent/mild steatosis: Grades 0, 1 and 2; Advanced steatosis: Grade 3; Absent/mild NASH: Grades 0, 1 and 2; Advanced NASH: Grade 3; Absent/mild fibrosis: Grades 0, 1 and 2; Advanced fibrosis: Grades 3 and 4. NASH: Nonalcoholic steatohepatitis.

**Table 4 Severity of nonalcoholic fatty liver disease and serum levels of micronutrients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Vitamin D, pg/mL** | **Vitamin B12, pg/mL** | **Zinc, mg/L** | **Iron, mcg/dL** | **Magnesium, mg/dL** |
| Steatosis | *r* | -0.120 | 0.095 | 0.58 | 0.007 | -0.053 |
|  | *P* value | 0.042 | 0.047 | 0.359 | 0.879 | 0.468 |
| NASH | *r* | -0.126 | -0.012 | -0.018 | 0.030 | -0.078 |
|  | *P* value | 0.034 | 0.812 | 0.775 | 0.553 | 0.291 |
| Fibrosis | *r* | -0.061 | 0.095 | -0.029 | 0.031 | -0.080 |
|  | *P* value | 0.303 | 0.048 | 0.647 | 0.538 | 0.272 |

Spearman’s correlation. NASH: Nonalcoholic steatohepatitis.