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Trabajo Original

Pediatría

Serum glutathione peroxidase is associated with nonalcoholic fatty liver disease in children and adolescents

La glutatión-peroxidasa sérica se asocia a la enfermedad del hígado graso no alcohólico en niños y adolescentes

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Abstract

Background and aims: oxidative stress is an important factor in the pathophysiology of non-alcoholic fatty liver disease (NAFLD). This study aimed to compare the serum levels of malondialdehyde (MDA), glutathione peroxidase (GPx) and antioxidant micronutrients in children and adolescents with and without NAFLD.

Methods: a cross-sectional study with patients between 8-18 years old, of both sexes. Diagnosis of NAFLD: presence of steatosis on ultrasound and absence of history of ethanol consumption and other liver diseases. Anthropometric measures, MDA, GPx, Interleukin-6, serum levels of vitamins A, C and E, selenium, zinc, and copper were evaluated.

Results: eighty-nine children with mean age of 12 (3) years, 57.3 % female and 24 % with NAFLD were evaluated. Those with NAFLD had more frequent abdominal obesity (high waist-height ratio: 81.0 % x 48.5 %; $p = 0.009$). After logistic regression NAFLD was associated with high body mass index/age (p-adjusted = 0.021) and with reduced serum GPx (p-adjusted = 0.034). There was a positive correlation between MDA and copper (r = 0.288; $p = 0.006$), IL-6 (r = 0.357; $p = 0.003$) and a negative one with vitamin A (r = -0.270; $p = 0.011$).

Conclusions: oxidative stress is present in children with NAFLD and non-invasive markers such as GPx and BMI can be used in clinical practice and help in the early screening of NAFLD.

Keywords:

Non-alcoholic fatty liver disease. Malondialdehyde. Glutathione peroxidase. Antioxidant micronutrients. Children. Adolescents.

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Data availability statement: the data is private because it belongs to human beings. However, if needed, databases can be made available in the future.

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Resumen

Antecedentes y objetivos: el estrés oxidativo es un factor importante en la fisiopatología de la enfermedad del hígado graso no alcohólico (EHGNA). Este estudio tuvo como objetivo comparar los niveles séricos de malondialdehído (MDA), glutatión-peroxidasa (GPx) y micronutrientes antioxidantes en niños y adolescentes con y sin NAFLD.

Métodos: estudio transversal con pacientes de 8-18 años de ambos sexos. Diagnóstico de NAFLD: presencia de esteatosis en la ecografía y ausencia de antecedentes de consumo de etanol y otras enfermedades hepáticas. Se evaluaron medidas antropométricas, MDA, GPx, interleucina-6, niveles séricos de vitaminas A, C y E, selenio, zinc y cobre.

Resultados: se evaluaron 89 niños con edad media de 12 (3) años, 57,3 % del sexo femenino y 24 % con EHGNA. Aquellos con EHGNA tuvieron obesidad abdominal con mayor frecuencia (relación cintura-altura alta: 81,0 % x 48,5 %; $p = 0,009$). Después de la regresión logística, la EHGNA se asoció con un índice de masa corporal/edad elevado (p ajustado = 0,021) y con una GPx sérica reducida (p ajustado = 0,034). Hubo correlación positiva entre MDA y cobre (r = 0,288; $p = 0,006$), IL-6 (r = 0,357; $p = 0,003$) y negativa con vitamina A (r = -0,270; $p = 0,011$).

Conclusiones: el estrés oxidativo está presente en niños con NAFLD y los marcadores no invasivos como GPx e IMC pueden usarse en la práctica clínica y ayudar en la detección temprana de NAFLD.

Palabras clave:

Enfermedad del hígado graso no alcohólico. Malondialdehído. Peróxido de glutatión. Micronutrientes antioxidantes. Niños. Adolescentes.

INTRODUCTION

Non-alcoholic fatty liver disease is characterized by the accumulation of fat in the hepatocytes of individuals without a history of chronic ethanol consumption or other liver diseases (1,2).

Considered the most common chronic liver condition today, it has also become the target of investigation in the child population, as it has a growing and estimated prevalence among children and adolescents at 3 % to 11 %, ranging from 46 % to 80 % in obese children (3).

In this population, of children and adolescents, the disease is usually asymptomatic, being diagnosed by an incidental evaluation of liver enzymes, evidence of steatosis on routine ultrasound or the occurrence of some extrahepatic manifestation (4).

Obesity, especially central obesity, and insulin resistance are risk factors for NAFLD, which is considered a hepatic manifestation of the metabolic syndrome and is associated with oxidative stress (OxS) and important cardiometabolic burden in childhood (5).

The pathogenesis of NAFLD is not fully understood, but it is known that important factors such as OxS induced inflammation with lipid peroxidation, cytokine activation and excessive production of reactive oxygen species (ROS) are associated with the progression of the disease in adults and children (6).

Oxidative stress occurs when there is an imbalance between the concentrations of pro and antioxidant species. In individuals with NAFLD, there may be an increase in serum OxS byproducts such as the malondialdehyde (MDA) marker, or impaired redox balance, also demonstrated by a decrease in glutathione peroxidase (GPx) levels and possibly stores of antioxidant micronutrients, of which vitamins C and E seem to act as protectors against OxS (7-9).

The pediatric population deserves special attention. Due to their life expectancy, they have a longer exposure to risk factors for NAFLD and consequently a greater probability of developing steatohepatitis, liver fibrosis, cirrhosis, and even hepatocellular carcinoma. Early diagnosis and the adoption of measures to prevent the progression of the disease will reduce damage in the future (7,8).

So, the objective of the present study was to compare the serum levels of MDA, GPx and antioxidant micronutrients in children and adolescents with and without NAFLD.

MATERIALS AND METHODS

STUDY DESIGN AND POPULATION

Cross-sectional study with children and adolescents followed up at a pediatric out clinic. The study was approved by the Research Ethics Committee under process number 1.471.817.

Patients of both sexes and aged between 8 and 18 years were included. Children under 8 years of age were not included because in the literature there are reports of liver diseases in this group associated with liver syndromes or autoimmune diseases. The non-inclusion criteria were patients with thinness according to body mass index (BMI)/age; consumption > 140 g of ethanol/week for males and > 70 g of ethanol/week for females; syndromic obesity, pubertal abnormalities; use of drugs that interfere with hepatic and glycemic metabolism such as corticosteroids, sulfa drugs, antipsychotics or amphetamines, antioxidant supplementation. Patients with hypothyroidism, other liver diseases (viral hepatitis A, B and C, autoimmune diseases, Wilson's disease, and hemochromatosis), presence of systemic infectious process in and/or acute in the last 10 days also were not included.

CLINICAL EVALUATION

Sociodemographic variables (age, sex, and family income), lifestyle habits (practice of physical activity (10)) and clinical data such as pubertal stage (11) and presence of acanthosis nigricans (12) were collected using a structured questionnaire and classified according to the references cited.

NAFLD CRITERIA DIAGNOSIS

Presence of hepatic steatosis on ultrasound of the upper abdomen, associated with alcohol consumption ≤ 140 g of ethanol/week for men and ≤ 70 g of ethanol/week for women, and in the absence of liver diseases such as B and C viruses, autoimmune, metabolic, and toxic (2). Abdominal ultrasonography was performed by a single evaluator.

ANTHROPOMETRIC ASSESSMENT

Anthropometric measurements were standardized and tested to determine statistical similarity. The value used was the average of 2 evaluators, and the maximum difference accepted between them was 0.5 cm. Weight was measured on a LD 1050 digital scale®, and height was measured on an LD 1050 stadiometer®, with a scale at 0.1 cm intervals. Anthropometric nutritional status was assessed according to the BMI/age and sex indicator, (13) using the WHO AnthroPlus, 2011 version 16. To classify body weight adequacy, patients were divided into two groups: adequate weight for age when $-2 < z\ score < +1$ and overweight when $z\ score > +1$. Waist circumference (WC), measured at the minimum circumference between the iliac crest and ribcage (14) and neck circumference (NC), measured at the midpoint of the neck (15), were measured and evaluated. Waist/height ratio (WHR) was considered normal when ≤ 0.5 (16). The Conicity Index (CI) ranged from 1.14 to 1.16 for children younger than 9 years and from 1.06 to 1.12 for children older than 10 years (17). The conicity index was calculated as follows:

Conicity index = waist circumference / (0.109 x square root of weight / height)

where waist circumference and height were measured in meters and weight in kg.

BIOCHEMICAL ASSESSMENT

Biochemical tests were performed after an 8-hour fast. Alanine aminotransferase (ALT), aspartate transaminase (AST) and gamma glutamyl transpeptidase (GGT) were analyzed using the dry chemistry method and had their suitability assessed according to reference values suggested by NASPGHAN (2017) (9). Fasting blood glucose and serum insulin values were evaluated according to the recommendations of the Brazilian Society of Diabetes, 2019-2020 (18). The HOMA-IR (Homeostatic Model Assessment) was analyzed based on the suggested value for insulin resistance (IR) ≤ 3.0, by Yan et al. 2013 (19). And the lipid profile, total cholesterol and fractions and triglycerides were based on the normality values of the Brazilian Society of Cardiology, 2019-2020 (20).

MDA was evaluated by thiobarbituric acid calorimetry and spectrophotometry, and GPx was evaluated by spectrophotometry, with reference values of 2.3-4.0 µmol/L and 4171-10881 U/L, respectively. IL-6 was evaluated by chemiluminescence and with a normal value < 3.4 pg/ml.

Vitamins C, E and A, selenium, zinc, and copper levels were measured in the blood and for the analysis of the adequacy of these markers the values considered acceptable were those suggested by the laboratory, being 4.6-15.0 mg/dl, 3-10, 0 mg/dl, 0.3-0.7 mg/dl, 20.0-190.0 µg/dl, 70.0-120.0 µg/dl and 80.0-160.0 µg/dl, respectively.

STATISTICAL ANALYSIS

The statistical program Statistical Package for the Social Science® (SPSS) version 17.0 was used for data analysis.

To calculate the sample size, it was considered a difference of 6 % (21) in the prevalence of oxidative stress in the groups with and without NAFLD, a power of 80 % and alpha of 5 %. Thus, the number needed to answer the objective of the study was eighty-six patients.

Categorical variables were expressed as absolute and relative frequency, and quantitative variables as mean and standard deviation (SD). The sample was divided in two groups with and without NAFLD. The following statistical tests were used to compare groups: t-test for independent samples to compare quantitative variables and chi-square and Fisher's exact test to compare categorical variables. Correlations tests of Pearson and Spearman were used to test linear association between the variables. P values < 0.05 were considered statistically significant. It was performed a logistic regression analysis to investigate confounding. The variables included in the model were those that showed a *p*-value ≤ 0.20 in bivariate analysis (high BMI/age, elevated MDA, reduced GPx and low copper).

RESULTS

Eighty-nine children and adolescents were included in the study aged between 8 to 18 years and 50 (56 %) were female. For comparison purposes the sample was divided into two groups with (24 %) and without (76 %) non-alcoholic fatty liver disease. It was observed that the group with NAFLD had a higher percentage of individuals with high body mass index/age, high neck circumference and high waist height ratio (Table I).

As for laboratory findings the group with NAFLD had a higher frequency of high total cholesterol and low-density lipoprotein cholesterol, but when all types of dyslipidemias were analyzed together, there was no differences between the groups. There were also no differences between them regarding liver enzymes, micronutrients, and vitamins. However, it was observed that individuals with NAFLD had more frequently reduced levels of GPx (23.8 % x 2.9 %; $p = 0.007$) and elevated levels of MDA (19.0 % x 5.9 %; $p = 0.085$), although this last difference did not reach statistical significance (Table II).

To assess confounding variables, it was performed a logistic regression analysis with the variables: high BMI/age, elevated MDA, reduced GPx and low copper. After adjustment, the associations between NAFLD and high BMI/age (p adjusted = 0.021) and reduced glutathione peroxidase (p adjusted = 0.034) remained, however the same did not happen with the associations between elevated malondialdehyde (p adjusted = 0.205) and low copper (p adjusted = 0.118).

Table III shows the correlations between serum levels of malondialdehyde, glutathione peroxidase and antioxidant micronutrients and vitamins. There were positive and statistically significant correlations between serum levels of MDA and copper ($r = 0.288$; $p = 0.006$) and IL-6 ($r = 0.357$; $p = 0.003$) and negative with vitamin A ($r = -0.270$; $p = 0.011$) and GPx ($r = -0.250$; $p = 0.018$). There were no statistically significant correlations between GPx, antioxidant micronutrients and vitamins.

Table I. Demographic and anthropometric characteristics of children and adolescents with and without non-alcoholic fatty liver disease followed up at the Pediatrics Outpatient Clinic, 2021

Characteristics	NAFLD		*p*-value
	Yes 21 (24 %)	No 68 (76 %)	
Age (years)*	12 (2)	12 (3)	0.924
Male sex	11 (52.4 %)	28 (41.2 %)	0.366
Physical activity practice	9 (42.9 %)	20 (29.4 %)	0.251
Presence of acanthosis nigricans	12 (57.1 %)	40 (58.8 %)	0.891
Pubertal stage			NA
Pre-puberty	3 (14.3 %)	17 (25.0 %)	
Pubescent	16 (76.2 %)	45 (66.2 %)	
Post pubescent	2 (9.5 %)	6 (8.8 %)	
High body mass index/age	20 (95.2 %)	39 (57.4 %)	*0.001*
High neck circumference	17 (81.0 %)	26 (38.2 %)	*0.001*
High waist-height ratio	17 (81.0 %)	33 (48.5 %)	*0.009*
High waist circumference	18 (85.7 %)	45 (66.2 %)	0.085
High conicity index	20 (95.2 %)	67 (98.5 %)	0.418

**Mean (standard deviation); NA: not applicable.*

Table II. Laboratorial characteristics of children and adolescents with and without non-alcoholic fatty liver disease followed up at the Pediatrics Outpatient Clinic, 2021

Characteristics	NAFLD		*p*-value
	Yes 21 (24 %)	No 68 (76 %)	
Elevated ALT	0 (0.0 %)	2 (2.9 %)	1.000
Elevated AST	1 (4.8 %)	2 (2.9 %)	0.559
Elevated GGT	1 (4.8 %)	0 (0.0 %)	0.236
High total cholesterol	11 (52.4 %)	17 (25.0 %)	*0.018*
High LDL- cholesterol	9 (42.9 %)	14 (20.6 %)	*0.042*
Low HDL- cholesterol	12 (57.1 %)	23 (33.8 %)	0.056
Elevated triglycerides	14 (66.7 %)	33 (48.5 %)	0.146
Dyslipidemia	16 (76.2 %)	48 (70.6 %)	0.618
High fasting glucose	1 (4.8 %)	3 (4.4 %)	1.000
HOMA-IR elevated	10 (47.6 %)	24 (35.5 %)	0.310
Elevated MDA	4 (19.0 %)	4 (5.9 %)	0.085
Reduced GPx	5 (23.8 %)	2 (2.9 %)	*0.007*
Elevated IL-6*	3 (27.3 %)	16 (27.6 %)	1.000
Low copper	2 (9.5 %)	1 (1.5 %)	0.137
Low zinc	0 (0.0 %)	1 (1.5 %)	1.000
Low selenium	3 (14.3 %)	6 (8.8 %)	0.435
Low vitamin A	3 (14.3 %)	6 (8.8 %)	0.435
Low vitamin C	15 (71.4 %)	47 (69.1 %)	0.840
Low vitamin E	3 (14.3 %)	9 (13.2 %)	1.000

**Data referring to 69 patients. ALT: alanine aminotransferase; AST: aspartate transaminase; GGT: gamma glutamyl transpeptidase; LDL-cholesterol: low density lipoprotein cholesterol; HDL-cholesterol: high density lipoprotein cholesterol; HOMA-IR: Homeostatic Model Assessment; MDA: malondialdehyde; GPx: glutathione peroxidase; Interleukin-6: IL-6.*

Table III. Correlation between serum levels of malondialdehyde, glutathione peroxidase and antioxidant micronutrients in children and adolescents followed up at the Pediatrics Outpatient Clinic, 2021

Antioxidant micronutrients	Malondialdehyde r (*p*-value)	Glutathione peroxidase r (*p*-value)
Serum copper	*0.288 (0.006)*	-0.051 (0.636)
Serum zinc	-0.115 (0.284)	0.054 (0.615)
Serum selenium	0.001 (0.994)	-0.063 (0.560)
Vitamin A	*-0.270 (0.011)*	-0.013 (0.903)
Vitamin C	-0.179 (0.093)	0.109 (0.309)
Vitamin E	0.085 (0.429)	-0.061 (0.569)
Interleukin-6*	*0.357 (0.003)*	-0.169 (0.166)

DISCUSSION

Our results showed that children and adolescents with non-alcoholic fatty liver disease more often have higher body mass index for age, increased neck circumference and waist-to-height ratio and low levels of glutathione peroxidase when compared to those without NAFLD. This study also showed a positive correlation between malondialdehyde and serum levels of copper and IL-6 and a negative correlation with vitamin A and glutathione peroxidase.

The interrelationship between genetic, epigenetic, and environmental factors supports the pathogenesis and progressive liver damage of NAFLD in children, which has aroused interest due to its relationship with cardiovascular and metabolic impairment in childhood or even in adulthood (22,23).

Obesity is directly and independently associated with NAFLD and its increased prevalence and severity. Consequently, obesity also appears to increase liver-specific mortality, even in children (24). It seems that this connection occurs when hepatocytes store excess lipids, mainly in the form of triglycerides, initiating hepatic steatosis. That is, the excess of circulating free fatty acids resulting from exacerbated lipolysis and the decrease in the uptake of these free fatty acids by the subcutaneous adipose tissue, promotes the accumulation of fat, including in the liver (24).

Thus, high BMI status is recognized as a risk factor for NAFLD and the prevalence of NAFLD is 10 to 20 times higher in obese children and adolescents compared to lean ones (8). However, it is known that central obesity is more associated with NAFLD than total body fat (25). In one study, where obese children aged 10 to 18 years were evaluated, NAFLD was more frequent in patients with high weight and neck circumference, which in turn evaluate central fat accumulation and can even be used as predictors of NAFLD (25). In our sample, the increase in BMI, WHR and NC were associated with NAFLD, pointing to the high presence of total body fat and the increase in central body fat as an associated factor for the disease.

The explanation for central body fat being more associated with NAFLD seems to lie in the fact that visceral adipocytes, which are more lipolytic than subcutaneous ones, store and mobilize triglycerides faster than those in other regions, increasing the supply of free fatty acids in the portal system, which stimulate gluconeogenesis and inhibit hepatic insulin clearance, contributing to elevated blood glucose and insulin levels and thus the development of insulin resistance (IR) (26,27).

Thus, obesity and IR seem to compose the first steps in the development of non-alcoholic fatty liver disease, without, however, all the pathogenesis and evolution of NAFLD to steatohepatitis, liver fibrosis, cirrhosis, and even hepatocellular carcinoma, with the possible absence of cirrhosis in children, being completely established (28).

What has been widely presented and discussed is that in the presence of obesity and alterations in the mitochondria, there is an increase in oxidative stress and in the production of pro-inflammatory cytokines in patients with liver disease (29).

The greatest production of reactive oxygen species occurs in the mitochondria and for this 1-2 % of the oxygen existing there is consumed. Under normal conditions and amounts, ROS act as signaling molecules and their unnecessary production is minimized through non-enzymatic and enzymatic antioxidant mechanisms (30).

In the persistent presence of hepatic steatosis, there may be triggering of the oxidative stress process that is characterized in NAFLD by mitochondrial dysfunction, increased release of inflammatory mediators, increased production of oxidative stress byproducts, such as the MDA marker, or impaired redox balance, as well demonstrated by a decrease in GPx levels, or even by impaired inactivation of ROS by deficient reserves of antioxidant nutrients (4,22).

Glutathione peroxidase is one of the most potent parts of the enzymatic defense system against free radical accumulation. Its action takes place by controlling the levels of hydrogen peroxide and lipid hydroperoxides, reducing them to alcohols and water, respectively, which can be subsequently detoxified and removed from the body or, in the case of water, used in other processes (30). Compromised GPx metabolism and activities of antioxidant enzymes in the blood were also observed in the present study. According to the findings of our study, patients with NAFLD showed an increased state of OxS, evidenced in lower serum levels of GPx, when compared to the values of individuals without NAFLD.

In a study developed by Nobili et al. (2005) (31), carried out with children with and without NAFLD, where the blood profile of various forms of glutathione and the presence of systemic oxidative stress were evaluated, there was impairment of glutathione metabolism

and activity of antioxidant enzymes, according to observed serum values in patients with NAFLD. A similar result was observed when comparing children with and without NAFLD in a study that tested GPx as a marker of oxidative stress (32).

Our results showed that the evaluation of the decrease in GPx was significantly associated with NAFLD, ie, patients with NAFLD had an increased state of OxS, evidenced in lower serum levels of GPx, when compared to the values of individuals without NAFLD. These findings reinforce the existence of a decline in the enzymatic antioxidant defense system and the possibility that GPx is a marker, regardless of the degree of steatosis and disease progression (33).

The presence of hepatic steatosis and oxidative stress trigger an increase in the reserve of MDA, which can be used as a standard measure to determine the degree of cellular oxidation (8,34). MDA is a secondary product of lipid peroxidation, derived from the interruption of the endocyclization of polyunsaturated fatty acids, such as linoleic, arachidonic, and docosahexaenoic acids (35).

Although we found a trend towards higher MDA values in children with NAFLD, showing that there may be evidence of changes in the degree of cellular oxidation in this sample, the assessment of the adequacy of this marker was not associated with the disease. This result was contrary to the study published by Bell et al. (2011) (34), where there was a significant increase in hepatic MDA in children with NAFLD when compared with control children or children with chronic hepatitis C virus. According to another study with a similar objective, MDA increases with the severity of NAFLD (30), which may explain our results, since most of our sample had mild cases of the disease.

In the present study, a negative correlation between MDA and GPx was observed. That is, in case of increased levels of MDA, due to the exacerbation of OxS, there is a decrease in the levels of the antioxidant enzyme GPx, portraying the body's attempt to promote defense against the accumulation of free radicals (30).

The pathogenesis of NAFLD has an inflammatory factor characterized mainly by an increase in tumor necrosis factor alpha (TNF-α) and a decrease in adiponectin. TNF-α increases the formation of reactive oxygen species and promotes hepatocyte apoptosis and liver inflammation, while adiponectin decreases the accumulation of hepatic triglycerides (36). TNF-α is also responsible for the increase in lipolysis and consequent increase in the flow of free fatty acids and their accumulation in the liver by suppressing insulin kinase receptor activity and decreasing glucose uptake causing hyperinsulinemia and by promoting IL-6 expression which reduces insulin receptor substrate expression (36).

In the sample studied here, we found a positive correlation between levels of MDA and IL-6. Literature suggests that IL-6 is increased in patients with NAFLD (3,29); however, this expression seems to be higher in individuals with increased OxS process, with NASH or more severe liver disease (37). Ahadi et al. (2021) (38), in a review on NALFD in obese and non-obese individuals, states that the increase in IL-6 in this population is more linked to the degree of visceral fat accumulation than to the accumulation of subcutaneous fat. If we consider that our sample seems to have a higher degree of OxS, represented by the consumption of glutathione peroxidase, we can explain the existence of this correlation.

Through their lipoprotective, antioxidant, antifibrotic and immunomodulatory functions, vitamins and minerals play a role in the pathogenesis of NAFLD (28).

Micronutrients such as vitamin A, C and E, copper, selenium, and zinc, exert their function as non-enzymatic antioxidants, inhibit the oxidation of biomolecules, neutralizing the harmful effects of oxidation caused by free radicals, thus ensuring redox homeostasis (8).

Our analyzes showed that the decrease in serum levels of copper, selenium, vitamins A and C was more frequent in those with NAFLD, but without statistical significance. It is noteworthy that, despite the absence of statistical significance, this result is interesting because there is a possibility that the disease is related, either as a cause or effect, with suspension of detoxification in the liver in the absence of non-enzymatic antioxidants (8,35,36).

Also, among the correlation analyzes, a negative correlation was identified between MDA and vitamin A. This result can be corroborated by the fact that carotenoids, once converted into vitamin A, can benefit the liver through its antioxidant function and in regulating the expression of genes involved in inflammation and lipid metabolism. Or even because, if in the presence or elevation of oxidative stress, this same serum concentration of vitamin A can be reduced (28).

In addition to the previous correlations, a positive correlation was also seen between MDA and serum copper reserves. According to Mendonza et al. (2017) (39), which determined whether lower tissue copper is associated with increased NAFLD severity in children, & Antonucci et al. (2017) (40), who described the role of copper and copper-binding antioxidant compounds against NAFLD in the development and progression of NAFLD, patients with NAFLD had lower serum levels of copper and this deficiency promotes changes in lipid metabolism, insulin resistance and negative regulation of mitochondrial beta-oxidation, alterations related to increased oxidative stress and the pathophysiology of the disease under study.

Limitations of this work were the measurement of only two markers of oxidative stress and the use of ultrasound. Performing a biopsy for diagnosing NAFLD in this study would be unethical, as this is an invasive test. The use of ultrasound is justified, therefore, as it is a non-invasive, inexpensive, accessible, and widely used method to assess the presence of NAFLD in children. As a strong point of the research, we can mention the public of children and adolescents, and the fact that patients with and without overweight or obesity were evaluated, characteristics that are not frequent in previous studies.

In conclusion, when comparing children with NAFLD and without NAFLD, it was observed that the first group showed an association between the studied disease and a higher body mass index/age, higher neck circumference, and higher waist/height ratio. There was also an association between NAFLD and lower adequacy of serum levels of glutathione peroxidase. The results also showed an impairment of the defence process against oxidative stress in the body of children and adolescents with NAFLD and that non-invasive markers, such as GPx and BMI, can be used in clinical practice and help in the early screening of the disease.

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