Obese Boys Wıth Low Concentratıons of Hıgh Densıty Lıpoproteın Cholesterol are at Greater Rısk of Hepatosteatosıs

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Abstract

Purpose: Non-alcoholic fatty liver disease (NAFLD) and associated morbidities have become a public health problem due to a global three-fold increase in incidence among obese children over the last three decades. Although the gold standard for diagnosis of NAFLD is liver biopsy, it is not widely used in children. Imaging techniques, including magnetic resonance and ultrasound, can provide information on liver fat deposition, with variable sensitivity. Therefore, a number of other predictors are being investigated for pediatric screening and diagnostic purposes. The aim of this study was to assess easily measured parameters to prompt further investigation for NAFLD in obese children. Methods: Obese children/adolescents with a Body Mass Index (BMI) percentile >95 were enrolled in the study (n=353). After a 12-hour fast, venous glucose, insulin, cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and uric acid were measured and full blood count was performed in all subjects. The TG/LDL ratio, the AST/platelet ratio index (APRI score) and the Homeostatic Model of Assessment-Insulin Resistance (HOMA-IR) were calculated. All patients underwent abdominal ultrasound examination to assess heaptosteatosis. Results: Of 353 patients, median age 12.5 (range: 6-17.9) years, 210 (59%) patients had US-proven hepatosteatosis. Female gender reduced the risk of steatosis 2.08 fold (p=0.005), one unit increase in HDL reduced the risk of steatosis 1.02 fold (p=0.042) and one unit increase in the BMI led to a 1.11 fold (p=0.002) increase in the risk of steatosis. Conclusion: Gender, BMI and HDL were found to be predictors of steatosis. Male patients with low HDL and high BMI are at greater risk of steatosis and should be carefully examined for the presence of NAFLD.

Keywords: *Fatty liver, childhood, high density lipoprotein, , hepatosteatosis, obesity*

Introductıon

Non-alcoholic fatty liver disease (NAFLD) is a wide-spectrum metabolic condition characterized by the accumulation of fat in at least 5% of the hepatocytes. It begins as an inflammatory process with steatohepatitis and can progress to fibrosis and end-stage liver disease cirrhosis. Globally, NAFLD is the most frequent cause of chronic liver disease. It is usually associated with obesity, insulin resistance, metabolic syndrome and dyslipidemia (1).

Since definitive diagnosis requires a liver biopsy, which may be associated with increased morbidity and is thus often avoided in pediatric patients, prevalence of the condition among children is unclear. Estimates of the prevalence of fatty liver in obese children has ranged up to 77% while the prevalence of histologically proven NAFLD among obese children in the USA is 38%, the frequency among the normal population is 9% [1,2]. Ethnic, genetic and environmental factors are known to play a role in the development of NAFLD and it is more frequently observed among pubertal males and Hispanic men [3-5]. The pathogenesis of NAFLD may be explained by the well established double-hit hypothesis. The first hit is insulin resistance which leads fat to accumulate in the liver, resulting in increased free oxygen radicals, the second hit, leading to steatohepatitis.

Assessment of liver fat deposition

Magnetic resonance imaging (MRI) can detect hepatosteatosis when fat deposition ≥5% while ultrasound, which is widely used in pediatric patients, has low sensitivity in cases where the fat build up is below 20% [4,5]. A quantitative elastographic ultrasound technique is still under development [5].
Some scoring systems consisting of clinical and biochemical parameters are also used. The non-invasive fibrosis grading score, the aspartate aminotransferase to Alanine aminotransferase (AST)/ALT ratio), AST/platelet ratio and the Fibrosis 4 calculator (FIB-4 using the formula: Age x AST /Platelet x √ALT) score are also in use as non-invasive markers for NAFLD [6,8]. Recently, a model constructed using gamma glutamyl transferase (GGT), Alkaline Phosphatase (ALP) and platelets has also been evaluated [8], though it requires further development for use in routine practice. In our study, routinely used biochemical and hormonal markers and anthropometric measurements were assessed in order to determine a good, non-invasive marker for the diagnosis of NAFLD.

Materıals And Methods

All children and adolescents, aged between 6 and 18 years, attending the pediatric endocrinology outpatients clinic over a two year period (2014-2015) with obesity (as defined by Kurtoglu *et al* [9]; see below) were eligible for this prospective study. Exclusion criteria were: monogenic obesity, type 2 diabetes, patients with secondary obesity syndromes and acute or chronic disease. In addition any patient with an underlying endocrinologic disease and/or those under medication were excluded from the study.
The height and weight of the children included in the study were measured using standard measuring techniques and the same combined stadiometer/weighing scale (Seca 703 sensitive to 1 mm and accurate to 100 g ; SecaGmbH&Co KG, Hamburg, Germany) for all subjects.. The Body Mass Index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters (weight [kg]/height squared [m2]). The children whose BMI was above the 95th percentile, according to the age, gender and ethnicity were classified as obese for the purpose of this study, as previously described by Kurtoglu *et al* [9].
Following a 12-hour overnight fast, venous blood samples were drawn into plain tubes in the morning. LDL, TG, HDL, total cholesterol, VLDL, glucose and uric acid were tested using a Roche kit on the autoanalyzer. The insulin and thyroid hormone levels were measured by chemiluminescence, using a Bio-DPC kit and the Immulite 2000 device. Complete blood count was measure by automated system.
Oral glucose tolerance test was conducted using 1.75 g glucose/kg with a maximum glucose dose of 75 g.). Subjects with HOMA-IR >3.16 were accepted to be insulin resistant, as previously described by Sahin *et al* [10]. Cumulative total insulin was calculated as previously described [9]. Briefly, the measured insulin at each time point in the OGTT was added together for each patient. If this cumulative value, hereafter referred to as “total insulin”, exceeded 300 μU/ml then the patient was considered to have hyperinsulinemia. Those with fasting HDL levels ≤40 mg/dL and fasting TG levels ≥110 mg/dL were considered dyslipidemic [3,4].
All the patients underwent abdominal ultrasound examinations, by the same radiologist, using the SSA-660A Xario Toshiba ultrasound device, (Toshiba Inc., Tokyo, Japan) with a 3.5MHz convex probe for hepatobiliary ultrasound. US-proven hepatosteatosis was graded as follows: Grade 0: Normal parenchymal liver echogenicity by comparison with the right renal cortex [5]; Grade I: Mild diffuse increase in echogenicity. The diaphragm and the intrahepatic blood vessel walls appear normal. Grade II: Moderate increase in echogenicity. The diaphragm and the intrahepatic blood vessel walls are slightly obscured. Grade III: Distinct increase in echogenicity. The diaphragm, intrahepatic blood vessel walls and the posterior view of the right lobe are severely or totally obscured.

Results

Table 1. Distribution of descriptive characteristics between the patients with or without steatosis

	US-proven Steatosis		P**
	No (n=143)	Yes (n=210)	
Age (years)	12 (6-17.3)	13 (6-17.9)	**0.004**
Gender			
Male	48 (29.8)	113 (70.2)	**<0.001**
Female	95 (49.5)	97 (50.5)	
Birth weight (grams)	3260 (1200-6000)	3300 (1200-5700)	0.286
BMI-SDS	2.49 (1.30-7.90)	2.63 (.120-8.78)	**0.002**

*Continuous variables are presented as "median (min-max)", while the categorical variables are presented as "number (percentage)".

** Pearson's Chi-Square Test or Fisher's Exact Test test was used to compare the patients with and without US-proven steatosis as appropriate.

Body mass Index (BMI)

Table 2. The laboratory results, HOMA-IR values and OGTT status of the study subjects with or without US-proven steatosis

	US proven steatosis		p**
	No (n=143)	Yes (n=210)	
TSH (µIU/ml)	2.20 (0.68-7.97)	2.12 (0.50-11.80)	0.627
Free T4 (µg/dL)	1.06 (0.30-1.88)	1.04 (0.48-1.50)	0.467
Uric Acid (mg/dL)	4.8 (2.5-7.8)	5.4 (2.9-10.0)	**<0.001**
AST (U/L)	20 (11-52)	23.7 (9.9-93.0)	**<0.001**
ALT (U/L)	17 (8-131)	24.4 (7.6-179.0)	**<0.001**
AST/ALT	1.16 (0.40-2.11)	0.92 (0.46-2.89)	**<0.001**
Platelet count (/µL)	319000 (30000-3730000)	310000 (38100-3930000)	0.568
APRI Score	0.006 (0.001-0.137)	0.007 (0.001-0.050)	**<0.001**
HDL (mg/dL)	47 (24.5-92.0)	43 (24.9-86.0)	**0.001**
LDL (mg/dL)	97 (42.3-207.0)	99 (42-339)	0.988
TG (mg/dL)	97 (42-265)	104.8 (31.3-516.0)	**0.027**

TG/HDL	2.11 (0.47-7.08)	2.61 (0.49-16.65)	**0.001**
VLDL (mg/dL)	19 (8.5-53.0)	22 (6.2-103.0)	**0.019**
FBG (mg/dL)	89.8 (73.0-11.1)	88.8 (70.9-158.0)	0.207
Insulin (µIU/ml)	13.6 (2.0-74.5)	18.3 (2-72)	**<0.001**
HOMA-IR	2.98 (0.42-19.50)	4.03 (0.45-26.53)	**<0.001**
OGTT status			
Not tested n(%)	47 (35.6)	85 (64.4)	**0.035**
Tested n(%)	34 (23.9)	108 (76.1)	
Total Insulin (n=142)	485 (109-1198)	481 (114-3745)	0.624
<300 n(%)	9 (47.4)	10 (52.6)	**0.018**
≥300 n(%)	25 (20.3)	98 (79.7)	

*Continuous variables are presented as "median (min-max)", while the categorical variables are presented as "number (percentage)".

****Mann Whitney-U Test was used to compare the measurement data**

Thyroid stimulating hormone (TSH), Fasting blood glucose (FBG), Aspartate Aminotransferase (AST), Alanin Aminotransferase (ALT), Triglycerides (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL), AST/Platelet ratio index (APRI score), Homeostatic Model of Assessment-Insulin Resistance (Homa-IR), Oral glucose tolerance test (OGTT)

Table 3. The logistic regression analysis evaluating the efficiency of a range of factors in predicting steatosis.

	OR	95% CI	p*
Age	1.021	0.908-1.150	0.725
Gender**	0.481	0.288-0.802	**0.005**
BMI	1.109	1.040-1.183	**0.002**
Uric Acid	1.201	0.943-0.1.530	0.138
AST	1.046	0.980-0.1.116	0.175
ALT	1.011	0.978-0.1.046	0.514
HDL	0.977	0.955-0.991	**0.042**
Triglycerides	0.994	0.972-0.1.016	0.571

VLDL	1.042	0.933-0.1.164	0.464
HOMA-IR	0.986	0.892-0.1.090	0.787

OR: Odds ratio; CI: Confidence interval

***The logistic regression analysis was used for prediction and Hosmer-Lemeshow Test was used for model fit**

**Male to female

Body mass Index (BMI), Triglycerides (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL), AST/Platelet ratio index (APRI score), Homeostatic Model of Assessment-Insulin Resistance (Homa-IR), Oral glucose tolerance test (OGTT).

Table 4. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the variables BMI and HDL according to the predetermined cut-off values

	Cut-Off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
BMI(kg/m^2)	25.05	87.1	25.9	63.3	57.8
	29.05	62.4	61.5	70.4	52.7
HDL (mg/dL)	47.02	64.8	47.6	64.5	47.9
	49.05	71.4	36.4	62.2	46.4

%: Percentage

Cross table was used for detecting the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)

Body mass Index (BMI)), High density lipoprotein (HDL)

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