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Clinical and Biochemical Predictors of Non-Alcoholic Fatty Liver Disease in Obese Children and Adolescents

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Abstract

Background: Obesity and its associated comorbidities are growing worldwide, including non-alcoholic fatty liver disease (NAFLD), which is one of the leading causes of chronic liver diseases in both children and adults. The aim of this study was to determine the prevalence of NAFLD among obese children and to investigate the clinical and biochemical predictors associated with NAFLD.

Methods: Ninety obese children and adolescents aged 12-18 years were enrolled in this study. All participants underwent anthropometric measurements and biochemical analyses including fasting blood glucose, serum insulin, serum triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and liver function tests. Ultrasonography was used to diagnose NAFLD.

Results: The prevalence of NAFLD was 38.9% among obese children, 68.6% of whom met the criteria for metabolic syndrome. Children with NAFLD had significantly higher body mass index, waist circumference, ALT, total cholesterol, LDL-c, TG, fasting insulin, and lower HDL-c levels than those with normal liver ultrasound ($P< 0.05$). Insulin resistance was significantly more common in the NAFLD group (88.6% vs. 18.2%) ($P<001$). Logistic regression analysis revealed that BMI and HOMA-IR were independent predictors of NAFLD ($P = 0.034$ and 0.022, respectively).

Conclusion: More than one-third of obese children have NAFLD, which is closely linked to metabolic syndrome and insulin resistance.

Key Words: Insulin resistance, Metabolic syndrome, Non-alcoholic fatty liver disease, Obese children.

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1- INTRODUCTION

Childhood obesity has recently increased globally, affecting both developed and developing countries. Excess adiposity in childhood is associated with acute and long-term health problems, including an increased risk of hypertension, type 2 diabetes, and cardiovascular illnesses, as well as an increased likelihood of premature mortality and middle-age morbidity, regardless of adult weight status (1).

Non-alcoholic fatty liver disease (NAFLD) is one of the main causes of chronic liver disorders in both adults and children. This disease affects the liver in a broad range of ways, the mildest of which is plain fatty liver (hepatic steatosis). However, there is a potentially severe form of non-alcoholic steatohepatitis (NASH) that is characterized by inflammation of the liver and, in some cases, the development of advanced fibrosis and cirrhosis (2). Pediatric nonalcoholic fatty liver disease is strongly associated with obesity; patients with nonalcoholic fatty liver disease have an increased risk of developing type II diabetes, dyslipidemia, hypertension, insulin resistance (IR), metabolic syndrome (MetS), and cardiovascular diseases in adulthood. Additionally, children in these categories face an increased risk of developing chronic liver disease and subsequent liver failure (3).

Patients with NAFLD have non-specific symptoms or are frequently asymptomatic, making early detection extremely difficult. The gold standard for the diagnosis in adults is liver biopsy. However, owing to the procedure's invasive nature, it is not recommended for children and is generally avoided (4). In 2012, the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) advised that "all obese children aged 10 years and older should be screened for liver disorders by liver function tests and liver ultrasonography as a first diagnostic step" (5).

This study aimed to assess the clinical predictors and biochemical profiles of NAFLD among obese children and identify high-risk groups.

2- MATERIALS and METHODS

2-1. Study design and population

This cross-sectional study included 90 obese children aged 12-18 years referring to Pediatric Endocrinology Unit, Suez Canal University Hospital, Egypt. The study subjects were divided into two groups: obese children with NAFLD (n=35) and obese children without NAFLD (n=55), based on the ultrasonographic evidence of fatty liver.

2-2. Inclusion and exclusion criteria

The inclusion criteria were as follows: children and adolescents with body mass index (BMI) ≥ 95th percentile and 12-18 years of age. Additionally, the exclusion criteria included the following: patients with known disorders causing fatty liver such as hepatitis B virus (HBV) and hepatitis C virus (HCV), Wilson's disease, glycogen storage disease, and type 1 diabetes, as well as those having long-term uses of drugs known to cause steatosis (e.g., glucocorticoids and aspirin), and any case of syndromic obesity patients with renal failure, recent trauma, or acute illness.

2-3. Sample analysis

2-3-1. Anthropometric measurements

Weight, height, and waist circumference (WC) were recorded in all children. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (in meters). All records were plotted on the Egyptian growth charts. Obesity was defined as a BMI equal to or above the 95th percentile using the criteria from the International Obesity Task Force (IOTF) adjusted for age and sex (6). Waist

circumference was measured at the level of the umbilicus, and abdominal obesity was defined as WC ≥ 90th percentile for age and sex (7).

Height and weight were measured in all subjects; BMI was calculated as weight in kilograms divided by height (in meters squared). Obesity was defined according to the criteria from the International Obesity Task Force (IOTF) if BMI ≥ 95th percentile for age and sex (6). Waist circumference (WC) measured at the level of the umbilicus and values ≥ 90th percentile was considered abdominal obesity (7).

2-3-2. Pubertal development

Based on Tanner stages, pubertal development was classified into three phases, prepubertal (Tanner 1), pubertal (Tanner 2-4), and post-pubertal (Tanner 5) (8, 9).

2-3-3. Blood pressure

Using the clinical learning guide for calculating blood pressure, blood pressure readings were placed on the percentile (10). Hypertension was defined as systolic or diastolic pressure of ≥ 90th percentile for age and sex (11).

2-3-4. Biochemical tests

All patients were subjected to the following tests (following not less than 8 h fasting period): triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein cholesterol (HDL-c), fasting blood sugar (FBS), aspartate aminotransferase (AST), and alanine aminotransferase (ALT); the measurements were carried out using an automated chemistry analyzer (COBAS e411 Roche Diagnostics, Germany). TG was considered high if > 150 mg/dl, TC and LDL were considered elevated if > 200 and 130 mg/dl, respectively, low HDL-c diagnosed if value < 40 mg/dl, high fasting blood sugar value >100 mg/dl, and upper cutoff points of both ALT and AST at 41 and 37 U/l, respectively (12).

Fasting serum insulin hormone levels were measured by ELISA (Immunospec Corporation, CA, USA) and insulin sensitivity index: HOMA-IR (homeostasis model assessment) was calculated according to the following formula: HOMA-IR= [fasting insulin (µU/mL) × fasting glucose (mg/dL)]/405. HOMA-IR ≥ 3.16 was interpreted as impaired insulin sensitivity or insulin resistance (13).

2-3-5. Liver ultrasound

Ultrasonography (US) was used to diagnose NAFLD using the Philips ClearVue 650 ultrasound system by the same radiologist who was blinded to the clinical and biochemical data of the patients. Liver steatosis was classified as follows: absent steatosis was identified as normal liver echotexture; mild steatosis (grade I) as diffuse increase in hepatic echogenicity; moderate steatosis (grade II) as moderate and diffuse increase in parenchymal echoes with moderately impaired visualization of vessel walls and diaphragm; and severe steatosis (grade III) as small echoes with absent visualization of hepatic vessels, diaphragm, and posterior part of the right lobe (14).

2-3-6. Metabolic syndrome

Metabolic syndrome (MetS) was diagnosed if the patients had three or more of the following modified criteria according to the International Diabetes Federation (IDF) consensus report:

1) Fasting blood glucose levels ≥100 mg/dl

2) Blood pressure ≥ 90th percentile

3) WC ≥ 90th percentile or adult cutoff if lower

4) TG levels ≥ 150 mg/dl

5) HDL-c ≤ 40 mg/dl (15)

2-4. Statistical analysis

The statistical evaluations were carried out using SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA). Data was summarized as mean ± standard deviation (SD). Comparisons between different groups in the present study were performed using Student's t-test for comparing continuous data when normally distributed and the Mann-Whitney U test when not normally distributed. Furthermore, to compare categorical data, the chi-square (x2) test was performed. Statistical significance was set at $p < 0.05$. Multiple regression analysis was used to evaluate independent predictors of NAFLD in obese children. Receiver operating characteristic (ROC) curves were generated to identify the cut-off values, sensitivity, and specificity for significant parameters associated with NAFLD.

2-5. Ethical consideration

Signed written informed consent was obtained from the parents of each patient. The study was approved by the institutional research ethics committee of the Faculty of Medicine, Suez Canal University (IRB-3126).

3- RESULTS

A total of 90 obese children were enrolled in this study; 29 (32.2%) were males and 61 (67.8%) were females, with a mean age of 15.1±2.9 years and mean BMI of 29±4.2 kg/m^2. Thirty-two (35.5%) children fulfilled the criteria for metabolic syndrome. Furthermore, 35 (38.9%) were diagnosed with NAFLD according to the ultrasonographic features; most of them were grade I or II 32 (91.4%).

3-1. Clinical Parameters

In the NAFLD group (13 males, 22 females), the mean age was 15.1±2.2 years, as compared to 15.2±2.1 years in the non-NAFLD group (16 males, 39 females). There was no statistically significant difference in age or sex (P=0.24, P=0.46).

The mean BMI was significantly higher in patients with NAFLD (31.2±2.6) kg/m^2 than in patients without NAFLD (27±2.2) kg/m^2. Furthermore, the mean WC of the NAFLD group was 86.2±6.89 cm, whereas the non-NAFLD group had a mean WC of 71.60±3.67 cm. The difference was statistically significant between the two groups, with a P value of 0.0027 for BMI and P=<0.001 for WC.

Puberty was found in half of the patients with NAFLD compared to 43.6% of the non-NAFLD group. However, the pubertal stage distribution wasn't significantly different between the NAFLD and non-NAFLD groups (**Fig. 1**).

Although the mean BP was within the normal range, it was significantly higher in the obese group with NAFLD than in the obese group without NAFLD; this was statistically significant (P<0.05). Acanthosis nigricans was present in 60% of NAFLD patients (**Table 1**).

3-2. Biochemical parameters

Children with NAFLD had significantly higher total cholesterol, triglyceride, fasting blood sugar, fasting serum insulin, and lower HDL-c levels than those with normal liver ultrasound (P<0.05) (**Table 1**).

The calculated HOMA-IR was 6.4±2.6 and 3.6±1.8 for the NAFLD and non-NAFLD groups, respectively. This finding suggests that insulin resistance is significantly higher in patients with NAFLD (31) (88.6%) than in patients without NAFLD (10) ([18.2%]; p<0.001). Receiver operating characteristic (ROC) analysis was performed to obtain the cut-off values of HOMA-IR for predicting a higher risk for NAFLD, with an area under the curve (AUC) of 0.865 (95% CI 0.784-0.946), and a cut-off of 3.15, sensitivity of 88.6%, and specificity of 72.7% (**Fig. 2**).

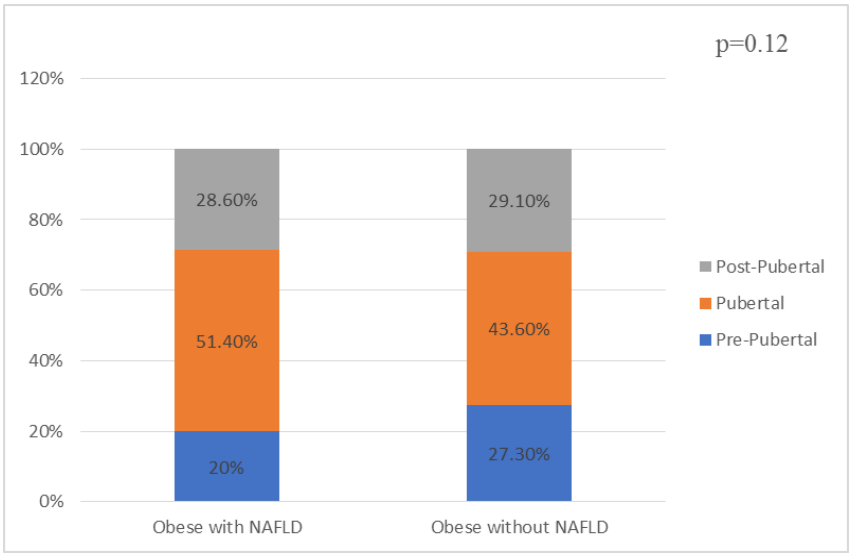

Fig. 1: Associations of pubertal development by Tanner stage with NAFLD in obese children

Table-1: Clinical and biochemical characteristics in obese children with and without NAFLD

Parameter	Obese with NAFLD "n=35" Mean ± SD or n (%)	Obese without NAFLD "n=55" Mean ± SD or n (%)	P value
Age (years)	15.1 ± 2.2	15.2 ± 2.1	0.2442
Sex (M/F)	13/22	16/39	0.4641
BMI (kg/m2)	31.2±2.6	27±2.2	0.0027*
WC (cm)	86.20± 6.89	71.60 ± 3.67	0.001*
Systolic BP (mm Hg) Diastolic BP (mm Hg)	121.8±9.6 74.4±6.2	106±8.7 68.6±5.6	0.031 0.042
Acanthosis Nigricans	21 (60)	32 (58.2)	0.6246
AST (U/l)	39.86± 9.2	31.4±7.4	0.0901
ALT (U/l)	46.3 ± 8.9	33.1 ± 10.2	0.007*
Total cholesterol (mg/dl)	198.1 ± 24	187.6 ± 20.3	0.0291*
Triglyceride (mg/dl)	126.2 ± 28.7	104.9± 33	0.0021*
LDL cholesterol (mg/dl)	131.6 ± 25	125.4 ± 23	0.2361
HDL cholesterol (mg/dl)	39 ± 8.3	51.2 ± 7	0.001*
Fasting blood sugar (mg/dl)	99.3 ± 19.2	89.2 ± 10.7	0.002*
Fasting serum insulin (µU/ml)	36.4 ± 18.6	10.8 ± 5.2	0.001*
HOMA-IR	6.4±2.6	3.6±1.8	0.001*
Insulin resistance HOMA-IR>3.16	31 (88.6)	10 (18.2)	0.001*
MetS	24 (68.6)	8 (14.5)	0.001*

* P < 0 05 (statistically significant). BMI, body mass index; WC, waist circumference; BP, blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; MetS, metabolic syndrome.

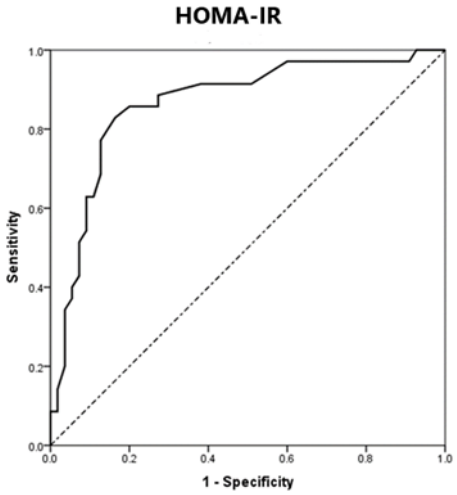

Fig 2: Receiver operating characteristic (ROC) curves to identify the cut-off value of homeostatic model assessment of insulin resistance HOMA-IR for association with non-alcoholic fatty liver disease in obese children.

Metabolic syndrome was diagnosed in 24 (68.6%) patients with NAFLD, as compared to eight (14.5%) in patients without NAFLD; the difference was statistically significant ($p<0.001$).

Obese children with NAFLD had a mean ALT of 46.3±8.9 U/l. For the non-NAFLD group, the mean ALT was 33.1±10.2 U/l. The difference between the two groups was statistically significant ($p=0.007$).

3-3. Logistic regression analysis for NAFLD predictors

Regression analysis was conducted to assess independent variables for NAFLD. Variables that have been found to be statistically significant, including BMI, WC, total cholesterol, triglyceride, HDL-c, ALT, FBS, HOMA-IR, and metabolic syndrome were considered as independent variables in the analysis. As shown in **Table 2**, the occurrence of NAFLD was significantly associated with BMI (B=0.761, P=0.034) and HOMA-IR (B=0.553, P=0.022).

4- DISCUSSION

In the current study, the prevalence of fatty liver was 38.9 % (n=35) among the 90 obese children and adolescents; most of them had a mild degree of fatty liver infiltration. This prevalence is lower than that reported by Jain et al. (62.5%) (16); however, it is consistent with the reports of a pediatric autopsy study conducted in 2006 by Schwimmer et al., where the NALFD prevalence ranged from 9.6% in normal weight subjects to 38% in obese children and adolescents (17). Nevertheless, the prevalence in various populations remains difficult to compare, as the published data differ in their study design, sample selection, diagnostic modality used to define fatty liver, and the age, sex, and ethnicity of the study population.

Both BMI and WC were shown to be significantly higher in the NAFLD group; moreover, an increased BMI was found to be a strong predictor of fatty liver disease. Hagström et al. found that having a high BMI as an adolescent increases the chance of developing a serious liver disease later in life by 5% for every 1 kg/m2 rise in BMI (18). Additionally, it was established that increasing abdominal obesity increases the chance of developing fatty liver as a result of hepatic lipid deposition (19); Dâmaso et al. demonstrated that

every 1 cm increase in waist circumference is associated with a twofold increase in the probability of developing NAFLD in obese children and adolescents (20).

Table-2: Logistic regression analysis for predictors of NAFLD in obese children

Predictors	B	S.E.	P value	OR	95% C.I	
					Lower	Upper
BMI	0.761	0.828	0.034*	2.172	0.034	0.872
WC	0.236	0.543	0.092	1.452	0.523	1.239
Total cholesterol	1.012	0.764	0.231	1.023	0.645	1.254
Triglyceride	0.465	0.032	0.674	1.213	0.854	2.543
HDL cholesterol	0.524	0.795	0.392	1.964	0.423	3.765
ALT	0.188	0.156	0.230	1.829	0.610	1.126
Fasting blood sugar	0.083	0.087	0.342	0.920	0.776	1.092
HOMA-IR	0.553	0.045	0.022*	3.981	0.287	2.311
MetS	0.602	0.067	0.092	2.134	1.076	1.123

* P < 0 05 (statistically significant). BMI, body mass index; WC, waist circumference; HDL, high-density lipoprotein; ALT, alanine aminotransferase; HOMA-IR, homeostatic model assessment of insulin resistance; MetS, metabolic syndrome.

In the present study, half of the patients with NAFLD were in the pubertal stage (Tanner 2-4). Insulin resistance begins to develop throughout puberty, making it a critical time for the initiation and progression of obesity and related comorbidities. As reported by Amiel et al., the young people in the puberty age have lower insulin sensitivity than the pre-pubertal children and adults (21). The mechanism for the observed correlations between pubertal phases and histologic features of NAFLD is not completely understood. In addition, it is difficult to address the underlying mechanisms without evaluating bio-physiological parameters of pubertal development. Animal studies suggest that alteration in sex hormones during puberty influences liver disease progression by modulating cell damage response mechanisms (22). In contrast to our findings, a study conducted in 2007 observed a prevalence of hepatic steatosis of 52% in obese prepubertal children (23). Significant differences in the methodologies used in previous studies to assess NAFLD in obese children may partly explain the conflicting results. Additionally, confounders such as age, gender, and duration of obesity are not appropriately considered.

In our study, when comparing individuals with NAFLD and those without, the pattern of pubertal stages was not found to be substantially different. Consistent with this result, Kurku et al. reported that obesity and the progression of NAFLD in boys were not seen to have influenced pubertal development (24).

In the present study, significant dyslipidemia (lower HDL-c, higher triglyceride, and total cholesterol levels) was observed in NAFLD obese children. In a multiethnic study on children with NAFLD and according to pediatric cutoff values, the prevalence of elevated triglyceride, non-HDL cholesterol, and low HDL-c was 77, 58, and 88%, respectively (25). In contrast to our findings, Gupta et al. showed that lipid levels were not

statistically different in the children with and without NAFLD (3).

In our study, we found that the calculated HOMA-IR score was significantly higher in the NAFLD group (6.4±2.6). It was a good predictor for NAFLD in multivariate logistic regression analysis, where 88.6% of our NAFLD patients had insulin resistance. Significant elevation of HOMA-IR in obese children with fatty liver disease has been reported in several studies (26, 27). Furthermore, Schwimmer et al. found a correlation between IR indicators (HOMA-IR and the Quantitative Insulin Sensitivity Check Index QUICKI) and the degree of liver disease in 43 children with biopsy-proven NAFLD (28). These observations were explained by the fact that IR increases lipolysis and free fatty acid inflow, resulting in TG deposition inside hepatocytes (29). Moreover, it leads to hepatic fibrosis by increasing oxidative stress and fatty acid β-oxidation [28]. In our study, the ROC analysis of HOMA-IR, with a cutoff value of 3.15, showed a sensitivity of 88.6% and specificity of 72.7% for predicting NAFLD. This was concordant with Salgado et al., who stated that HOMA-IR values above or equal to 2.0 or 2.5 showed enhanced diagnostic value in distinguishing non-alcoholic fatty liver disease carriers with a sensitivity of 85% and a specificity of 83%, from the control group individuals, with a sensitivity of 72% and a specificity of 94% (30). A recent joint European practice guideline for NAFLD (31) concluded: 'HOMA-IR provides a surrogate estimate of insulin resistance in persons without diabetes and can therefore be recommended'.

Metabolic syndrome was identified in 68.6 percent of the patients with NAFLD compared to 14.5 percent of patients without NAFLD; this is because, while NAFLD is not conventionally included in the MetS criteria, it is commonly regarded as the hepatic manifestation of metabolic syndrome. Children with MetS had a fivefold increased chance of acquiring NAFLD compared to obese children without MetS in a case-control study of 150 obese children with biopsy-proven NAFLD and 150 without (32). In addition, Fu et al. revealed that NAFLD developed in 82.6% of obese children with metabolic syndrome, meaning NAFLD might serve as an early predictor of metabolic syndrome (33). Another study on 254 patients with biopsy-confirmed NAFLD found that the presence of MetS is a strong predictor of not only the severity of hepatic steatosis, but also the degree of fibrosis (34).

ALT is the main and cheapest screening test for NAFLD. Serum aminotransferase levels are slightly elevated in individuals with NAFLD, however liver enzymes may be normal in up to 78% of patients (35). This is concordant with our results, which demonstrated that only 14.2% of children with NAFLD had abnormal ALT levels. However, we found that the mean ALT was significantly elevated in the NAFLD group (46.3±8.9). Kim et al. reported that a high ALT level was identified as the most critical factor in NAFLD risk. However, this parameter is not a definite NAFLD indicator, nor is high ALT frequently seen in NAFLD patients. A serum ALT level of more than 40 IU/L corresponds to an NAFLD probability of <0.6. This result shows that high ALT levels alone do not accurately predict NAFLL. In addition to serum aminotransferases, additional markers are necessary for the accurate evaluation of NAFLD (36).

One of the limitations of this study is that the diagnosis of NAFLD is based on ultrasound imaging, which is approved as a safe, inexpensive, and non-invasive test with a sensitivity ranging from 60% to 96% and a specificity ranging from 84% to 100% (5). However, it cannot replace liver biopsy as a gold standard for diagnosis, which unfortunately carries the risk of

complications and is often not accepted by the parents.

5- CONCLUSION

Based on our findings, we can conclude that more than one-third of obese children had a substantial incidence of non-alcoholic fatty liver, which is tightly linked with metabolic syndrome parameters and insulin resistance. HOMA-IR is a valuable predictor of hepatic steatosis in obese children. Screening for NAFLD should be a part of the evaluation for all obese children since this disorder can be prevented with dietary intervention and proper exercise.

6- CONFLICT OF INTERESTS STATEMENT

None

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