Frequency of non-alcoholic fatty liver disease in overweight/obese children and adults: Clinical, sonographic picture and biochemical assessment

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KEYWORDS
Non alcoholic fatty liver disease (NAFLD); Subcutaneous fat thickness (SFT); Visceral fat thickness (VFT); Ultrasonography

Abstract   Non-alcoholic fatty liver disease (NAFLD) is a condition defined by significant lipid accumulation (5–10%) in hepatic tissue in the absence of significant chronic alcohol consumption. We aim to detect frequency of fatty liver among overweight/obese adults and children and associated clinical; anthropological measures; biochemical; genetic and imaging studies. Eighty three consecutive adults and 72 children included in the study. All patients underwent clinical measurements of height, body weight, body mass index (BMI), waist and hip circumference. Biochemical investigations were done to all subjects including liver function tests; lipid profile; fasting blood glucose; insulin resistance (IR); high sensitivity C reactive protein (hs-CRP); adiponectin and genotyping of adiponectin genes. Abdominal ultrasonography was done to search for fatty liver; to measure subcutaneous fat thickness (SFT) and visceral fat thickness (VFT). Fatty liver was detected in 47 (65.3%) children and in 52 (62.7%) adults. Correlation analysis in both groups revealed that enlarged liver was highly positively correlated to age; BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP); waist circumference; hip circumference, subcutaneous fat thickness (SFT) and Visceral fat thickness (VFT), alanine aminotransferase (ALT), aspartate aminotransferase/alanine aminotransferase (AST/ALT). In addition in adults to fasting blood glucose, cholesterol, triglycerides (TG), low density lipoprotein (LDL), IR and hs-CRP. Homozygous T adiponectin
1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition defined by significant lipid accumulation (5-10%) in hepatic tissue in the absence of significant chronic alcohol consumption [1]. Most patients with NAFLD have increased liver fat content alone (simple steatosis), but others develop increasing hepatic inflammation known as nonalcoholic steatohepatitis (NASH), and up to 20% of patients reveal progressive hepatic fibrosis and may eventually develop cirrhosis or liver failure [1,2,25].

The great majority of NAFLD occurs in the setting of so-called metabolic syndrome (MS), in which insulin resistance (IR) plays a key role [11]. The prevalence of MS (22%) and of NAFLD (20%) in the general US population are amazingly similar [7]. These observations support that primary NAFLD is a hepatic complication of MS.

NAFLD is the most common cause of chronic liver disease, constituting a major risk factor for progression to liver failure, cirrhosis, and hepatocellular carcinoma [2,3,8]. Particularly alarming are the data showing that NAFLD has become the most common cause of liver disease in children [18].

The Third Report of National Cholesterol Education Program (Adult Treatment Panel III; ATP III) provides a working definition of metabolic syndrome (MS) [13] based on a combination of five categorical risk factors: central obesity, hypertension, hypertriglyceridemia, low levels of HDL-cholesterol, and hyperglycemia.

The importance of body fat topography to metabolic disease was first recognized more than 60 years ago [1]. Subsequently, truncal obesity as measured by waist circumference and the waist–hip ratio has been the focal point of most definitions of the metabolic syndrome.

The pathophysiology of NAFLD is complex and available data suggest that environmental factors such as diet, exercise, and/or toxins [14] are likely to be important in its causation.

The echogenicity of the normal liver equals or minimally exceeds that of the renal cortex or spleen. Intrahepatic vessels are sharply demarcated, and posterior aspects of the liver are well depicted. A genetic predisposition is indisputably present in NAFLD, and several candidate genes affecting glucose and lipid metabolism have been proposed [21].

Two common single-nucleotide polymorphisms (SNPs) in exon/intron two of the adiponectin gene (45TG and 276GT) have been associated with cardio-metabolic risk, with haplotypes 45TT and 276GT/TT carrying a higher risk of type 2 diabetes and cardiovascular disease in cross-sectional and prospective studies. Adiponectin enhances oxidation of free fatty acid (FFA) and triglyceride-rich lipoprotein (TRLP) metabolism independently of systemic inflammation or insulin action [16]. The liver takes up circulating FFAs in a dose-dependent fashion and low density lipoprotein (LDL) and remnants through the LDL-receptor and the liver related receptor protein (LRRP). Consistently, postprandial lipid storage contributes substantially to liver triglyceride (TG) pool in NAFLD and the magnitude of postprandial lipemia correlated with liver steatosis [29].

C-reactive protein (CRP) is an acute phase-reactant protein synthesized by the liver that is also elevated in chronic inflammatory states. CRP levels have been shown to be closely related to obesity, in particular central or visceral fat deposition. Elevated serum hs-CRP was reported to be a diagnostic tool or predictor of disease progression in patients with NAFLD [26].

The aim of this study was to determine the frequency of NAFLD in examined children and adults and the associated clinical; biochemical; genetic and imaging investigations.

2. Materials and methods

2.1. Patients

We performed a cross-sectional, proof of concept study on 83 consecutive adults and 72 children recruited from the liver, pediatric clinics, Medical Service Unit, National Research Center. Patients were enrolled in a study about new evidences for obesity. The study protocol was approved by the Human Ethics Committee of National Research Center, and written informed consent was obtained. Children and adults of both sexes were enrolled. Patients with any of the following criteria were excluded from the study: hepatobiliary diseases, chronic liver diseases including viral hepatitis malignancies, ascites, medications known to cause hepatic steatosis (such as estrogens, corticosteroids, amiodarone, and valproate; at present or within the last 2 years), inflammatory bowel disease, human immunodeficiency virus (HIV), chronic drug or alcohol abuse (more than 20 g/day).

2.2. Anthropometric measurements and clinical examination

All patients underwent complete physical examination including measurements of height, body weight, waist and hip circumference. Body mass index (BMI) was calculated according to equation: BMI = weight (kg)/height$^2$ (m).

Waist circumference (WC) was measured at the level midway between the lowest rib margin and the iliac crest and was plotted on American percentiles for waist circumference [19]. Hip circumference was measured at the widest level over the greater trochanters in a standing position by the same examiner; then calculation of W/H ratio was done. Blood pressure and heart rate were measured in the sitting position after adequate resting time.

2.3. Laboratory measurements

Blood samples after 12 h fasting were collected from all individuals by a sterile venepuncture and are divided as follows:
five milliliters blood in an EDTA containing tube for DNA extraction using DNA extraction kits (QIAGEN). Three milliliters blood left in the tubes and allowed to clot for 30 min before centrifugation for 10 min then the sera separated from the clotted samples are used for liver function tests (ALT, AST, Albumin, Bil T and D, Alkaline Ph), lipid profile (total cholesterol, HDL, LDL, VLDL and triglycerides) and fasting glucose were measured by Olympus AU 400 autoanalyser. Insulin and serum high sensitivity C reactive protein CRP (hs-CRP) were measured in immulite by chemiluminescence. Insulin resistance was calculated by the homeostasis model (HOMA-IR) using the following formula:

\[
\text{HOMA-IR} = \frac{\text{fasting insulin (mU/L) } \times \text{ plasma glucose (mmol/L)}}{22.5} \quad [20].
\]

Adiponectin was measured by ELISA technique.

2. Genotype analysis of adiponectin gene (two mutations)

Two milliliters blood in a tube containing EDTA to obtain a complete hemogram stored at −80 °C until analysis. Genomic DNA was isolated from blood of overweight, obese individuals and controls.

2.5. Extraction of DNA

This method is used for whole blood collected in Vacutainer® EDTA tubes. Use preferably the QIAamp Blood Kit (Cat. No. 51106; Qiagen Inc., Valencia, CA, http://www.qiagen.com).

The samples were equilibrated to room temperature.

a. Heat block was heated to 56 °C.

b. Buffer AW1, Buffer AW2, and QIAGEN protease were ensured that they had been prepared according to the instructions.

c. All centrifugation steps should be carried out at room temperature.

d. 200 µl of the whole blood yielded 3–12 µg of DNA.

2.6. Procedure

1. 200 µl whole blood was added to 20 µl QIAGEN protease, and 200 µl Buffer AL into a 1.5 ml low binding microcentrifuge tube (e.g., Cat. No. T6050G, Marsh Biomedical Products, Rochester, NY). Mixed by vortexing.

2. Incubated at 56 °C for 10 min.

3. Spinned down briefly to remove drops from the inside of the tube.

4. 200 µl of 96–100% ethanol was added and mixed by vortexing.

5. The mixture was carefully applied from step above to a QIAamp spin column. Centrifuged 1 min at The QIAamp spin column was carefully opened and added full speed (15,000g). The tube containing the filtrate was discarded. The DNA would be bound to the filters in the spin columns. Column was placed in a clean 2 ml collection tube.

6. QIAamp spin column was opened and 500 µl Buffer AW1 was added without hitting the rim. The cap was closed and centrifuged 1 min at full speed. QIAamp spin column was placed in a clean 2 ml collection tube.

7. The QIAamp spin column was carefully opened and added 500 µl Buffer AW2. The cap was closed and centrifuged the QIAamp column for 3 min at maximum speed.

8. The 2 ml collection tube containing the filtrate was discarded, the QIAamp spin column was placed in a new collection tube and spinned for 1 min to remove residual buffer AW2.

9. The spin column was placed in a clean 1.5 ml low binding microcentrifuge tube. Two hundred microliters Buffer AE or distilled water was added to the spin column. Incubated at room temperature for 5 min to elute the DNA. Centrifuged 1 min at full speed. One microliters of extracted DNA was used for a 50 µl PCR.

10. Stored at 4 °C.

2.7. Genotyping

The adiponectin 45 G polymorphism was genotyped by amplification of genomic DNA using the following primers: forward, 5'-GAGTAGAICTTGCTGGAGTG-3'; reverse, 5'-TATCGTAGAGGATCTGTG ATG-3'. The product was digested with MnlI (New England BioLabs Inc.), and the digestion products were resolved by electrophoresis in a 2% agarose gel. The adiponectin 276 T polymorphism was genotyped by amplification of genomic DNA using the following primers: forward, 5'-GGCTC TTTCATCACAGACC-3'; reverse, 5'-AGATGCAGCAA AGCCAAAGT-3'. The product was digested with SmaI (New England BioLabs Inc.), and the digestion products were resolved by electrophoresis in a 2% agarose gel [5].

2.8. Ultrasound examination

In addition to the routine abdominal ultrasound examination based on the clinical indication. Liver size was determined by measuring distance between upper and lower borders in mid clavicular line. Liver parenchyma was examined with sagittal as well as longitudinal guidance of the probe and completed by lateral and intercostals views. The examination of the liver was carried out in dorsal recumbence and left lateral position and in inspiration. The presence of steatosis was recognized as a marked increase in hepatic echogenicity, poor penetration of the posterior segment of the right lobe of the liver, and poor or no visualization of the hepatic vessels and diaphragm. The liver was assessed to be normal if the texture was homogeneous, exhibited fine-level echoes, or was minimally hyperechoic or isoechoic compared with normal renal cortex, and if there was no posterior attenuation of the ultrasound beam. Fatty liver may be diagnosed if liver echogenicity exceeds that of renal cortex and spleen and there is attenuation of the ultrasound wave, loss of definition of the diaphragm, and poor delineation of the intrahepatic architecture [17]. To avoid false-positive interpretations, fatty liver should not be considered present if only one or two of these criteria are fulfilled. Hepatomegaly was defined as a liver size above 155 mm measured as a subcostal diameter in the midclavicular line. Transverse scanning was performed to measure the maximum subcutaneous fat thickness (SFT) and visceral fat thickness (VFT). Both measures were obtained 1 cm above umbilicus in the midline of the
abdomen. Application of the transducer on the body surface was done without undue pressure that would alter the body layer contour and thickness. SFT was defined as the distance between the external face of the rectoabdominal muscle and the internal layer of the skin. VFT was defined as the distance between the anterior wall of the aorta and the internal layer of the rectoabdominal muscle perpendicular to the aorta [23].

2.9. Statistical analysis

Data were expressed as mean ± SD and percentages. Mean values between different groups were compared using one way ANOVA test. \( \chi^2 \) was used to study the pattern of distribution of different variables. Correlations were performed with Pearson standard linear regression analysis. The SPSS package for windows version 13 was used for the analysis. \( p \leq 0.05 \) was considered significant, \( p < 0.001 \) was considered highly significant and \( p > 0.05 \) was considered insignificant.

3. Results

The baseline characteristics of the studied subjects are presented in Table 1. The mean age was 10.8 (4–18) years in children, while in adults it was 36.3 (18–60) years. Most of adults were females 79.5% while in children 52.8% were females. Twenty four adults and 27 children have average body mass index BMI (20–25) and they were considered as controls of the study. Eight adults and 23 children were overweight BMI (25–30) while obesity BMI (\( \geq 30 \)) was detected among 51 adults and 22 children. Fatty liver was detected in 47 (65.3%) children and in 52 (62.7%) adults.

In children, comparison between controls, overweight and obese persons revealed that there were significant differences between them as regards age, systolic blood pressure (SBP), subcutaneous fat thickness (SFT) and visceral fat thickness (VFT) as shown in Table 2. In adults the same comparison revealed that there were significant differences between them as regards all studied variables except high density lipoprotein (HDL) as shown in Table 3.

We found that all fatty livers were enlarged with rounded borders in children and adults. Fatty livers showed different degrees of hyperechogenicity and blurred vasculatures. It was noticed that the larger the liver the more echogenicity and blurring of intrahepatic vasculatures.

Correlation analysis of liver size and the studied variables revealed that the enlarged liver was positively correlated to all variables except fasting blood glucose; lipids; adiponectin; GOT and IR in children. It was found that enlarged liver among children and adults was highly positively correlated to age; BMI; SFT; VFT; waist circumference; hip circumference; SBP and DBP (\( P = .0001 \)). Moreover, in adults; the enlarged liver was positively correlated to fasting blood glucose; lipids; adiponectin; GOT and IR. The degree of positive correlation between both children and adults was highly significant for age; BMI; SFT; VFT; waist circumference; hip circumference; SBP and DBP.

\( \chi^2 \) was used to study the pattern of distribution of different variables. Correlations were performed with Pearson standard linear regression analysis. The SPSS package for windows version 13 was used for the analysis. \( p \leq 0.05 \) was considered significant, \( p < 0.001 \) was considered highly significant and \( p > 0.05 \) was considered insignificant.

### Table 1: Clinical characteristics of the studied subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) mean ± SD</td>
<td>10.8 ± 3.5</td>
<td>36.3 ± 13.3</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males no (%)</td>
<td>34 (47.2)</td>
<td>17 (20.5)</td>
</tr>
<tr>
<td>Females no (%)</td>
<td>38 (52.8)</td>
<td>66 (79.5)</td>
</tr>
<tr>
<td>BMI (Wt/Ht^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (20–25) no (%)</td>
<td>27 (37.5)</td>
<td>24 (28.91)</td>
</tr>
<tr>
<td>Overweight (25–30) no (%)</td>
<td>23 (31.9)</td>
<td>8 (9.63)</td>
</tr>
<tr>
<td>Obese (&gt;30) no (%)</td>
<td>22 (30.6)</td>
<td>51 (61.45)</td>
</tr>
<tr>
<td>Waist C (cm) mean ± SD</td>
<td>87.0 ± 14.2</td>
<td>91.1 ± 19.3</td>
</tr>
<tr>
<td>Hip C (cm) mean ± SD</td>
<td>97.9 ± 17.8</td>
<td>112.2 ± 18.3</td>
</tr>
<tr>
<td>SFT (cm) mean ± SD</td>
<td>1.4 ± 0.5</td>
<td>1.5 ± 0.8</td>
</tr>
<tr>
<td>VFT (cm) mean ± SD</td>
<td>4.1 ± 1.4</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal no (%)</td>
<td>25 (34.7)</td>
<td>31 (37.3)</td>
</tr>
<tr>
<td>NAFLD no (%)</td>
<td>47 (65.3)</td>
<td>52 (62.7)</td>
</tr>
</tbody>
</table>

| Body mass index (BMI), waist circumference (Waist C), hip circumference (Hip C), subcutaneous fat thickness (SFT), visceral fat thickness (VFT). |

### Table 2: Comparison between average, overweight and obese children.

<table>
<thead>
<tr>
<th>Variables (mean ± SD)</th>
<th>Average BMI (20–25) N = 27</th>
<th>Overweight BMI (25–30) N = 23</th>
<th>Obese BMI (&gt;30) N = 22</th>
<th>( F )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP mmHg</td>
<td>102.0 ± 8.2</td>
<td>101.6 ± 11.2</td>
<td>108.9 ± 18.1</td>
<td>3.954</td>
<td>.02*</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>66.8 ± 6.5</td>
<td>64.5 ± 6.4</td>
<td>70.3 ± 12.0</td>
<td>2.550</td>
<td>NS</td>
</tr>
<tr>
<td>SFT (cm)</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>12.195</td>
<td>.0001**</td>
</tr>
<tr>
<td>VFT (cm)</td>
<td>3.1 ± 1.4</td>
<td>3.6 ± 1.6</td>
<td>5.3 ± 1.7</td>
<td>9.939</td>
<td>.0001**</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>173.8 ± 29.5</td>
<td>169.3 ± 31.2</td>
<td>168.7 ± 38.1</td>
<td>.223</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>98.2 ± 31.3</td>
<td>104.5 ± 42.6</td>
<td>117.5 ± 57.1</td>
<td>1.534</td>
<td>NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>40.7 ± 8.7</td>
<td>38.6 ± 6.9</td>
<td>40.6 ± 8.4</td>
<td>.302</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>114.7 ± 28.1</td>
<td>124.6 ± 28.6</td>
<td>112.9 ± 28.4</td>
<td>.695</td>
<td>NS</td>
</tr>
<tr>
<td>AST (IU/dl)</td>
<td>27.7 ± 11.7</td>
<td>23.4 ± 7.8</td>
<td>27.6 ± 28.1</td>
<td>.438</td>
<td>NS</td>
</tr>
<tr>
<td>ALT IU/dl</td>
<td>14.4 ± 9.8</td>
<td>13.8 ± 10.4</td>
<td>22.1 ± 24.5</td>
<td>2.311</td>
<td>NS</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>22.3 ± 33.1</td>
<td>23.5 ± 29.5</td>
<td>24.8 ± 34.08</td>
<td>2.977</td>
<td>NS</td>
</tr>
<tr>
<td>IR</td>
<td>.11 ± .06</td>
<td>.18 ± .13</td>
<td>.13 ± .19</td>
<td>2.977</td>
<td>NS</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>1.8 ± 3.9</td>
<td>2.4 ± 4.4</td>
<td>3.4 ± 5.4</td>
<td>1.091</td>
<td>NS</td>
</tr>
</tbody>
</table>

Systolic blood pressure (SBP), diastolic blood pressure (DBP), subcutaneous fat thickness (SFT), visceral fat thickness (VFT), high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate transaminase (AST), alanine transaminase (ALT), insulin resistance (IR) and high sensitivity c reactive protein (hs-CRP).

NS: no significant.

* \( p \) value is significant.

** \( p \) value is highly significant.
Fatty liver disease was found to be a component of metabolic syndrome, especially in adults this was evidenced by morbidity and mortality among patients with chronic HCV infection. Liver steatosis or steatohepatitis represents a co morbidity that accelerates progression of chronicity; morbidity and mortality among patients with chronic HCV infection.

Fatty liver disease was found to be a component of metabolic syndrome, especially in adults this was evidenced by the significant correlations between liver size and BMI; SBP; diastolic blood pressure (DBP); subcutaneous fat thickness (SFT); visceral fat thickness (VFT); high density lipoprotein (HDL); low density lipoprotein (LDL); aspartate transaminase (AST); alanine transaminase (ALT); insulin resistance (IR) and high sensitivity c reactive protein (hs-CRP).

The sonographic picture of fatty liver was recorded in 65.3% of children and in 62.7% of adults. These percentages were significant differences as regards mean values of SBP, liver size, adiponectin and hs-CRP as shown in Table 5. In adults, it was found that mean values of hs-CRP was 11.7 ± 17.6 mg/L higher among subjects with fatty liver than in persons with normal liver 3.8 ± 8.2 mg/L (P = .03, significant).

**Table 3** Comparison between average, overweight and obese adults.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP mmHg</td>
<td>107.9 ± 9.4</td>
<td>108 ± 6.0</td>
<td>121.6 ± 15.9</td>
<td>10.408</td>
<td>.0001**</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>69.7 ± 6.1</td>
<td>71.1 ± 3.3</td>
<td>78.8 ± 9.3</td>
<td>11.477</td>
<td>.0001**</td>
</tr>
<tr>
<td>SFT cm</td>
<td>.9 ± .9</td>
<td>1.3 ± 4</td>
<td>1.8 ± 6</td>
<td>14.551</td>
<td>.0001**</td>
</tr>
<tr>
<td>VFT cm</td>
<td>1.7 ± .8</td>
<td>3.1 ± 1.0</td>
<td>3.8 ± 1.3</td>
<td>25.501</td>
<td>.0001**</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>176.9 ± 26.4</td>
<td>196.6 ± 34.9</td>
<td>219.0 ± 46.6</td>
<td>8.958</td>
<td>.001**</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>75.0 ± 31.4</td>
<td>97.6 ± 34.5</td>
<td>122.5 ± 62.8</td>
<td>6.507</td>
<td>.0022**</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>44.5 ± 10.8</td>
<td>42.9 ± 11.7</td>
<td>45.3 ± 11.5</td>
<td>.92</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>118.0 ± 28.1</td>
<td>137.3 ± 25.9</td>
<td>149.1 ± 42.6</td>
<td>5.05</td>
<td>.011*</td>
</tr>
<tr>
<td>AST (IU/dl)</td>
<td>21.1 ± 10.1</td>
<td>20 ± 7.7</td>
<td>28.4 ± 23.3</td>
<td>1.4</td>
<td>NS</td>
</tr>
<tr>
<td>ALT (IU/dl)</td>
<td>9.3 ± 7.2</td>
<td>12 ± 5.9</td>
<td>20.6 ± 21.3</td>
<td>3.4</td>
<td>.0399**</td>
</tr>
<tr>
<td>Adiponectin μg/ml</td>
<td>45.13 ± 4.7</td>
<td>22.5 ± 12.4</td>
<td>10.04 ± 14.53</td>
<td>27.375</td>
<td>.001**</td>
</tr>
<tr>
<td>IR</td>
<td>1.1 ± .8</td>
<td>1.1 ± .65</td>
<td>2.7 ± .2</td>
<td>5.006</td>
<td>.011*</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>1.5 ± 1.4</td>
<td>6.8 ± 10.4</td>
<td>12.3 ± 18.0</td>
<td>4.173</td>
<td>.022**</td>
</tr>
</tbody>
</table>

Systolic blood pressure (SBP), diastolic blood pressure (DBP), subcutaneous fat thickness (SFT), visceral fat thickness (VFT), high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate transaminase (AST), alanine transaminase (ALT), insulin resistance (IR) and high sensitivity c reactive protein (hs-CRP). NS: no significant.

* p value is significant.

** p value is highly significant.

**Table 4** Correlations between liver size, adiponectin and all studied variables in children and adults.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Children N = 72</th>
<th>Adults N = 83</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P value</td>
</tr>
<tr>
<td>Age</td>
<td>.585 .0001**</td>
<td>.594 .0001**</td>
</tr>
<tr>
<td>BMI</td>
<td>.350 .001**</td>
<td>.647 .0001**</td>
</tr>
<tr>
<td>SBP</td>
<td>.468 .0001**</td>
<td>.453 .0001**</td>
</tr>
<tr>
<td>DBP</td>
<td>.315 .0001**</td>
<td>.478 .0001**</td>
</tr>
<tr>
<td>Waist</td>
<td>.431 .004*</td>
<td>.663 .0001**</td>
</tr>
<tr>
<td>Hip</td>
<td>.348 .024*</td>
<td>.691 .0001**</td>
</tr>
<tr>
<td>Waist/hip</td>
<td>.016 NS</td>
<td>.276 .011*</td>
</tr>
<tr>
<td>SFT</td>
<td>.520 .0001**</td>
<td>.543 .0001**</td>
</tr>
<tr>
<td>VFT</td>
<td>.310 .024*</td>
<td>.707 .0001**</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>.015 NS</td>
<td>.284 .014*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>.034 NS</td>
<td>.361 .001*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>.264 NS</td>
<td>.360 .001*</td>
</tr>
<tr>
<td>HDL</td>
<td>.237 NS</td>
<td>.058 NS</td>
</tr>
<tr>
<td>LDL</td>
<td>.163 NS</td>
<td>.312 .007*</td>
</tr>
<tr>
<td>AST</td>
<td>.129 NS</td>
<td>.188 NS</td>
</tr>
<tr>
<td>ALT</td>
<td>.284 .043*</td>
<td>.323 .004*</td>
</tr>
<tr>
<td>AST/ALT</td>
<td>.390 .0001**</td>
<td>.128 NS</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>.097 NS</td>
<td>.262 .037*</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>.207 NS</td>
<td>.321 .003*</td>
</tr>
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</table>

Body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), subcutaneous fat thickness (SFT), visceral fat thickness (VFT), high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate transaminase (AST), alanine transaminase (ALT), insulin resistance (IR) and high sensitivity c reactive protein (hs-CRP).

NS: no significant.

* p value is significant.

** p value is highly significant.

4. Discussion

The sonographic picture of fatty liver was recorded in 65.3% of children and in 62.7% of adults. These percentages were high providing that all studied subjects were apparently healthy. NAFLD is a health problem affecting Egyptian community. The size of this problem is not well determined. The disease is so dangerous because it is what the National Institutes of Health refers to as a “silent disease” [30]. Non alcoholic fatty liver disease develops over a long period of time, but many people experience few, if any, symptoms until the condition worsens to non alcoholic steatohepatitis (NASH) or cirrhosis. This can explain why in our study children group in contrast to adult one, liver size where significant enlarged while their laboratory parameters including FBG; lipid profiles and IR still (in children) not affected as in adult, as this condition need long period to give its bad derangements.

This disease must not be ignored specially in our country; Egypt is considered a highest endemic area for prevalence of HCV infection. Liver steatosis or steatohepatitis represents a co morbid condition that accelerates progression of chronicity; morbidity and mortality among patients with chronic HCV infection.
DBP; waist circumference; hip circumference; SFT; VFT; fasting blood glucose; IR; and lipids which are components of the metabolic syndrome.

It was noticed that percentage of fatty liver among children was more than that of obese children. This means that fatty liver not only associated with overt obesity but also just overweight children can have enlarged liver. Abdominal adipose tissue includes distinct anatomic depots, a subcutaneous fat depot and an intraabdominal fat depot, which can be divided into intraperitoneal and retroperitoneal depots [15]. The intraperitoneal fat depot, also known as visceral fat. Subcutaneous fat differs from visceral fat in that venous drainage from subcutaneous fat is directed into the systemic circulation, whereas venous drainage from visceral fat is directed into the portal vein. The metabolic products thus reach the liver directly and exercise a first-pass effect on liver metabolism [12,6]. It was hypothesized that visceral fat releases free fatty acids and adipokines and thereby exposes the liver to fat accumulation. It was found that size of liver in children was directly correlated to liver size in adults but it was not correlated to adiponectin in children. This was related to serum values of adiponectin, but this relation did not reach the significant value in adults. At the same time the fatty liver was not correlated to adiponectin in children. This was against previous studies which suggested that serum adiponectin as well as hepatic gene expression of adiponectin and its receptor are decreased and inversely related to the degree of liver steatosis, subjects can be identified who have very high and very low insulin resistance (IR) and high sensitivity C reactive protein (hs-CRP).

Table 5 Laboratory investigations of subjects according to adiponectin genotype at position +276.

<table>
<thead>
<tr>
<th>Variables mean ± SD</th>
<th>In children</th>
<th>T/G + T/T</th>
<th>P value</th>
<th>In adults</th>
<th>T/G + T/T</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/G</td>
<td></td>
<td></td>
<td>G/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.0 ± 3.0</td>
<td>14.6 ± 5.3</td>
<td>NS</td>
<td>44.5 ± 8.6</td>
<td>40.2 ± 12.5</td>
<td>NS</td>
</tr>
<tr>
<td>SBP mmHg</td>
<td>111.6 ± 10.3</td>
<td>93.3 ± 5.8</td>
<td>.01</td>
<td>123.1 ± 17.5</td>
<td>121.2 ± 13.7</td>
<td>NS</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>69.2 ± 10.8</td>
<td>61.7 ± 12.6</td>
<td>NS</td>
<td>78.8 ± 9.4</td>
<td>78.6 ± 7.9</td>
<td>NS</td>
</tr>
<tr>
<td>Waist C (cm)</td>
<td>97.0 ± 17.8</td>
<td>94.5 ± 9.2</td>
<td>NS</td>
<td>98.4 ± 12.2</td>
<td>103.8 ± 15.8</td>
<td>NS</td>
</tr>
<tr>
<td>Hip C (cm)</td>
<td>104.3 ± 22.7</td>
<td>102.0 ± 14.1</td>
<td>NS</td>
<td>121.4 ± 10.4</td>
<td>119.1 ± 17.4</td>
<td>NS</td>
</tr>
<tr>
<td>FBS (mmol/L)</td>
<td>5.1 ± 0.5</td>
<td>5.5 ± 0.2</td>
<td>NS</td>
<td>5.6 ± 1.3</td>
<td>5.9 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>168.8 ± 32.9</td>
<td>209.3 ± 22.6</td>
<td>NS</td>
<td>217.4 ± 45.0</td>
<td>227.0 ± 42.6</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>104.5 ± 34.8</td>
<td>145.0 ± 23.8</td>
<td>NS</td>
<td>115.9 ± 74.8</td>
<td>136.6 ± 64.2</td>
<td>NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>43.2 ± 9.3</td>
<td>44.3 ± 15.6</td>
<td>NS</td>
<td>44.2 ± 7.5</td>
<td>42.6 ± 11.7</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>104.8 ± 27.0</td>
<td>136.0 ± 13.0</td>
<td>NS</td>
<td>146.6 ± 34.7</td>
<td>157.0 ± 34.7</td>
<td>NS</td>
</tr>
<tr>
<td>AST (IU/dl)</td>
<td>34.1 ± 35.3</td>
<td>27.3 ± 6.6</td>
<td>NS</td>
<td>25.0 ± 15.5</td>
<td>23.0 ± 9.4</td>
<td>NS</td>
</tr>
<tr>
<td>ALT (IU/dl)</td>
<td>27.9 ± 31.3</td>
<td>33.7 ± 26.3</td>
<td>NS</td>
<td>23.5 ± 15.8</td>
<td>19.9 ± 6.6</td>
<td>NS</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>4.1 ± 5.0</td>
<td>12.6 ± 10.1</td>
<td>.03*</td>
<td>6.9 ± 9.2</td>
<td>11.9 ± 12.7</td>
<td>NS</td>
</tr>
<tr>
<td>Adiponectin µg/ml</td>
<td>26.7 ± 12.6</td>
<td>25.6 ± 11.0</td>
<td>.04*</td>
<td>22.4 ± 10.3</td>
<td>20.7 ± 9.6</td>
<td>NS</td>
</tr>
<tr>
<td>SFT (cm)</td>
<td>1.6 ± 0.6</td>
<td>2.1 ± 0.5</td>
<td>NS</td>
<td>2.1 ± 0.6</td>
<td>1.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>VFT (cm)</td>
<td>3.9 ± 2.0</td>
<td>3.7 ± 1.6</td>
<td>NS</td>
<td>4.3 ± 1.6</td>
<td>3.6 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Liver (cm)</td>
<td>13.6 ± 0.4</td>
<td>15.6 ± 0.6</td>
<td>.01*</td>
<td>16.8 ± 1.0</td>
<td>16.5 ± 1.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), subcutaneous fat thickness (SFT), visceral fat thickness (VFT), fasting blood sugar (FBS), high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate transaminase (AST), alanine transaminase (ALT), insulin resistance (IR) and high sensitivity C reactive protein (hs-CRP).

NS: no significant.
*p value is significant.

DBP; waist circumference; hip circumference; SFT; VFT; fasting blood glucose; IR; and lipids which are components of the metabolic syndrome.

It was noticed that percentage of fatty liver among children was more than that of obese children. This means that fatty liver not only associated with overt obesity but also just overweight children can have enlarged liver. Abdominal adipose tissue includes distinct anatomic depots, a subcutaneous fat depot and an intraabdominal fat depot, which can be divided into intraperitoneal and retroperitoneal depots [15]. The intraperitoneal fat depot, also known as visceral fat. Subcutaneous fat differs from visceral fat in that venous drainage from subcutaneous fat is directed into the systemic circulation, whereas venous drainage from visceral fat is directed into the portal vein. The metabolic products thus reach the liver directly and exercise a first-pass effect on liver metabolism [12,6]. It has been hypothesized that visceral fat releases free fatty acids and adipokines and thereby exposes the liver to fat accumulation. It was found that size of liver in children was directly correlated to waist, hip circumferences, SFT and VFT. This means that size of liver in children was directly correlated to waist, hip circumferences, SFT and VFT. This means that we cannot depend on BMI only to define obese persons and adipokines and thereby exposes the liver to fat accumulation. It was found that size of liver in children was directly correlated to waist, hip circumferences, SFT and VFT. This means that we cannot depend on BMI only to define obese persons but truncal obesity must be assessed to avoid associated problems like fatty liver disease.

Insulin resistance (IR) may be defined as altered metabolic condition in which higher than normal insulin levels are needed to achieve normal metabolic responses or normal insulin concentrations fail to achieve a normal metabolic response. In the current study, it was found that insulin resistance was directly correlated to liver size in adults but it was not correlated to enlarged liver in children. Non-alcoholic fatty liver disease represents a continuum of hepatic injuries, which progress from simple fatty liver (FL or HS) to hepatocellular ballooning degeneration, formation of Mallory bodies and fibrosis (NASH). Previous studies suggested that pathogenesis of NAFLD includes two hits: The first hit involves accumulation of triacylglycerol (TAG) in hepatocytes. It has also been recognized that HS in itself leads to hepatic IR by activating protein kinase-theta (PKC-θ) and Jun N-terminal kinase (JNK) [28,10], which interfere with tyrosine phosphorylation of insulin receptor substrate (IRS) IRS-1 and IRS-2 and impairs insulin action in hepatocytes [22]. Steatosis and IR can cause and potentiate each other creating a vicious cycle of metabolic dysfunction, so, we can ask which appears first and can lead to the other. This may explain why IR was not correlated to fatty liver in children once the presence of hepatic steatosis is established, progression to steatohepatitis involves a ‘second hit’ and oxidative stress is thought to play a key role as fatty liver is more susceptible to oxidative injury [10].

Although hepatic fat accumulation, both in animals and in humans, is strongly associated with a decrease in insulin sensitivity, a large variability in this relationship exists that cannot be explained by other parameters regulating insulin sensitivity such as overall obesity, body fat distribution, or circulating adipokines. In other words, for the same amount of hepatic steatosis, subjects can be identified who have very high and very low insulin resistance [28].

Unlike most adipocytokines, adiponectin is decreased in the setting of obesity. Adiponectin is an antidiabetic hormone that correlates with insulin sensitivity [9].

In the current study, we found that fatty liver was inversely related to serum values of adiponectin, but this relation did not reach the significant value in adults. At the same time the fatty liver was not correlated to adiponectin in children. This was against previous studies which suggested that serum adiponectin as well as hepatic gene expression of adiponectin and its receptors are decreased and inversely related to the degree of liver injury and they explained role of adiponectin in NAFLD as adiponectin directly counteracts the effects of TNF-α on insulin signaling and lipid metabolism [4]. Moreover, genotyping of adiponectin gene at position 45 revealed that all subjects
have G/G polymorphism while comparison of the studied clinical and biochemical parameters in relation to T/T, G/T and G/G genotypes of adiponectin gene at position 267 revealed no significant differences among adults. On the other hand, in children, mean values of SBP were significantly higher in relation to genotypes G/T and T/T than genotype G/G. Mean values of liver size were significantly higher in relation to genotypes G/T and T/T than genotype G/G. In accordance with Musso and his colleagues found that the 45TT and 276GT/TT genotypes were more prevalent in NAFLD patients than in controls and independently predicted the severity of liver disease in NASH [21].

In a study evaluating the validity of liver enzymes in detecting persons with fatty liver, the sensitivity of alanine aminotransferase (ALT) was higher than that of aspartate aminotransferase (AST) and γ-glutamyl transferase (γGT) [27]. This was confirmed by our finding about ALT which was significantly correlated to size of the liver in children and adults in agreement with study of Ruhl and Everhart, who concluded that central adiposity, hyperleptinemia, and hyperinsulinemia were the major determinants of the association of overweight with elevated serum ALT activity [24].

We found that mean values of hs-CRP was significantly higher among cases with fatty liver disease. This was agreed with study of Siramolpiwat et al., who concluded that serum hsCRP is associated with NASH in patients with NAFLD. And also can be use as non-invasive diagnostic and screening test of NASH especially when combined with metabolic syndrome parameters [26].

The most widely accepted and most successful treatment for fatty liver is dietary and lifestyle changes aimed at reducing fat in the body. Many times, dietary changes are all that are needed to reverse and control the condition [25].

5. Conclusion

NAFLD affects a substantial portion of adults and children; it is associated with the metabolic syndrome, which includes obesity, insulin resistance, hyperlipidemia, and hypertension. NAFLD is emerging as one of the most common liver disorders claiming urgent attention of the public, clinicians and researchers. Obesity is an epidemic health disorder and NAFLD can be a major health problem.

References