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Reduction of Plasma Adiponectin in Egyptian Obese Children with Nonalcoholic Fatty Liver Disease

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Authors' contributions

Author AAM designed the study, wrote the protocol and supervised the work. Author MAEG wrote the first draft of the manuscript and managed the selection of patients. Authors DMEM and DAED carried out all laboratories work. Author KKEG performed the statistical analysis. Author HS managed the selection of patients and managed the analyses of the study. Author AMAA managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Obesity, insulin resistance and dyslipidemia are the most significant risk factors of non-alcoholic fatty liver disease (NAFLD) in children, and a major cause of liver-related morbidity. The aim of this study was to evaluate the serum levels of adiponectin, leptin and fasting insulin in obese children with NAFLD to explore the role of adiponectin in the pathogenesis of this disease.
Materials and Methods: The fasting serum levels of adiponectin, leptin, glucose, insulin, ALT,

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AST, total bilirubin, direct bilirubin, albumin, alkaline phosphatase, creatinine, cholesterol, triglycerides, HDL, LDL, GGT and CRP were measured in a group of 50 NAFLD children after making ultrasonography and 40 other participants were considered as a control group with comparable age, sex and body-mass index.

Results: Plasma adiponectin was found significantly low in NAFLD children than its level in control group (3.23± 2.5 vs 11.0 ± 2.95 ng/dl). Moreover, NAFLD group had significantly higher insulin resistance, fasting insulin 11.4± 4.9 vs 4.7±3.1 mu/l levels in comparison with control group. Regarding serum leptin, there was no significant difference. An inverse correlation was observed between adiponectin and homeostatic model assessment (HOMA-IR), fasting insulin, leptin, triglycerides, ALT, AST, GGT and BMI.

Conclusion: This data supports a role for low circulating adiponectin value in the pathogenesis of NAFLD and its association with insulin resistance.

Keywords: Non-alcoholic fatty liver; adiponectin; leptin; obesity.

1. INTRODUCTION

At present, non alcoholic fatty liver disease (NAFLD) is identified as an important liver disease in children, occurring even in the very young [1]. The incidence in the general population is 2.6% but it increases to 53% in obese children; thus, NAFLD is expected to become the most common cause of pediatric chronic liver disease [2]. NAFLD prevalence is increasing, alongside obesity, essentially because of sedentary lifestyles and high caloric diets [3]. NAFLD is considered the hepatic manifestation of the metabolic syndrome, which is characterized by insulin resistance, visceral obesity, hypertension, dyslipidemia, and abnormalities of fasting serum glucose levels. Pediatric patients with NAFLD have been shown to exhibit higher levels of insulin, total cholesterol, triglycerides, low-density lipoprotein cholesterol, and fasting glucose, as well as higher blood pressure values than obese children without NAFLD. Moreover, children with metabolic syndrome are more likely to display NAFLD than those without metabolic syndrome [4]. NAFLD is characterized by fat accumulation in the liver (steatosis) and insulin resistance, influenced by genetic susceptibility, epigenetic mechanisms, a sedentary lifestyle, and high caloric diets [5]. Hepatic triglyceride accumulation results from increased delivery of free fatty acid to the liver, increased lipogenesis, and impaired fatty acid metabolism in hepatocytes. Hepatic fat accumulation has been shown to exacerbate insulin resistance by interfering with phosphorylation of insulin receptor substrates [6]. Adipocytes or inflammatory cells infiltrating the adipose tissue in insulin resistance are responsible for adipocytokine secretion [7]. The adiponectin is highly abundant in human serum and is secreted by adipose tissue in inverse proportion to the body mass index [8]. Adipocytokines, including adiponectin, leptin, resistin and tumor necrosis factor-alpha, also appear to be involved in the progression of simple steatosis to non alcoholic steatohepatitis [9].

NAFLD refers to a wide spectrum of liver abnormalities ranging from simple liver steatosis (fat accumulation in the liver) to steatohepatitis (non-alcoholic steatohepatitis NASH), which may be associated with fibrosis and progress to cirrhosis and end-stage liver disease (9). Cirrhosis in children is rare but is reported. NAFLD has no specific symptoms or signs but should be considered in obese children. NAFLD does not have a proven treatment. Weight loss with family based treatments is the most acceptable management. Exercise and an applicable diet with low glycemic index and appropriate calorie intake are preferred. Drugs are promising but not sufficient in children for today [10]. So, we aim in this study to assess the relationship between selected adipokines in non alcoholic fatty liver disease to explore its role in the pathogenesis of this disease in obese children.

2. MATERIALS AND METHODS

2.1 Study Population

The study was carried out prospectively with 50 obese children (mean BMI 34.6±5.5 , mean age 13±2.69, range 5-16, 30 boys and 20 girls) with a suspected liver disease (hepatomegaly and/or ultrasonographic liver brightness and/or increased ALT level), who were seen in pediatric clinic of El Sahel Teaching Hospital between August 2012 and October 2013; in comparison with 40 control subjects (15 male, 25 female)

with (mean BMI 25.8±4.6), mean age 9.05±3.2, range 6-15).

The diagnosis of NAFLD was based on the standard criteria accepted by the American Gastroenterology Association (AGA) by ultrasonographic findings of bright liver [11] that is defined as abnormally intense, high level echoes arising from the hepatic parenchyma, with amplitude similar to that of echoes arising from the diaphragm and elevation of serum ALT activity.

An informed consent was obtained from all patients' parents. The study was carried out in accordance with the principles of the Declaration of Helsinki, and its appendices, and local and national laws. Ethical committee approval was taken from El Sahel Teaching Hospital.

The viral hepatitis (hepatitis B virus-HBV, hepatitis C virus-HCV, cytomegalo virus-CMV), toxic, autoimmune (AIH) and metabolic liver diseases (Wilson disease, α-1 antitrypsin deficiency, cystic fibrosis) were excluded by ELISA technique for both HBs (HBV) Ag and HCV Ab using (Axiom GmbH Germany) and for CMVIgG using (Orgenium Laboratories Helsinki FINLAND) for both case and control groups.

The body mass index (BMI) was calculated as the weight (kilograms) divided by the height (meters) squared for all children.

2.2 Blood Sampling and Biochemical Assays

A blood sample was collected after overnight fasting for each subject in patient and control groups. portion of blood was allowed to clot and then centrifuged at 3500 g for 5 min to separate the serum used for assessment of serum level of total cholesterol, triglycerides, lipoproteins: high density (HDL) and low density (LDL) as well as standard liver tests including (total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), albumin) using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. & Trade-Giza,Egypt). Serum aliquots were stored at -80°C and thawed immediately before the measurements of adiponectin, leptin, and CRP levels. A diponectin, leptin, fasting insulin and C-reactive protein (CRP) were measured by ELISA technique.

ELISA is a solidphase enzyme-amplified sensitivity immunoassay performed on microtiter plate with a sensitivity of 0.1 ng/ ml and intra-assay 3.6%.

The insulin resistance was assessed from fasting insulin and fasting glucose by homeostasis model (HOMA-IR) [12].

HOMA-IR was calculated using the following formula (12):

HOMA-IR=fasting serum glucose (mg/dl) × fasting insulin (μU/ml)/405.

2.3 Laboratory Evaluations

Serum level of adiponectin was determined with a commercially available AssayMax Human Adiponectin ELISA kit (Cat. No: EA2500-1, Assaypro, St. Charles, MO, USA) with sensitivity 0.5 ng/ml, and intra-assay and inter-assay variations were 4.1% and 7.2%, respectively. This assay employs a quantitative sandwich enzyme immunoassay technique. Serum level of leptin was measured using the BioSource leptin ELISA kit (Cat. No: KAP2281; BioSource Europe S.A, Nivelles, Belgium). The levels of serum hs-CRP were determined by Accu-Bind ELISA Kit (Monobind Inc, USA). And serum insulin (detected by ELISA using commercial Human kit). All assays were performed in duplicates according to the manufacturer's instructions.

2.4 Statistical Analysis

Results are expressed as mean ±standard deviation (SD). Patient and control groups were compared by student's t-test for independent values with Bonferroni correction. Correlations between different variables were determined by logarithmic regression and multivariate analysis. P values less than 0.05 were considered significant.

3. RESULTS

Anthropometric, clinical and biochemical parameters are illustrated in Tables 1 and 2. Significant differences were observed in HDL, bilirubin (T, D), albumin, creatinine, ALP, T. cholesterol, triglyceride and Leptin. Also, fasting glucose, plasma insulin, HOMA, ALT, LDL, GGT and CRP, were found significantly higher in NAFLD patients than control subjects. Plasma adiponectin was significantly lower in NAFLD group than control group. When correlation

analysis was performed, adiponectin levels were inversely correlated with triglycerides (r=-0.348, P<0.0001), with insulin (r=-0.509, P<0.0001), with HOMA (-0.445, P<0.0001), with leptin (r=0.725, P<0.0001), with ALT (r=-0.534, P<0.0001), with AST (r=-554, P<0.0001), with GGT (r=-0.461, P<0.0001) and with BMI (r=-0.593, P<0.0001). At the same time leptin showed a positive correlation with ALT and BMI (r=0.548, P<0.0001 & r=0.612, P<0.0001) respectively. In NAFLD group, serum aminotransferases were moderately high with ALT being higher than AST and ALT/AST ratio = 1.14. Regarding to the correlations between between serum levels of Adiponectin and Insulin, HOMA, Leptin. There inversely correlations between serum levels and these paramters were detected (Figs.1-3).

4. DISCUSSION

Fatty liver is a growing health problem worldwide. The important risk factors for NAFLD in children are obesity and insulin resistance. In general, NAFLD has no specific symptoms but should be considered in obese children. The most common admission reason is slightly elevated transaminases or coincidentally noticed hepatomegaly, ALT being higher than AST.

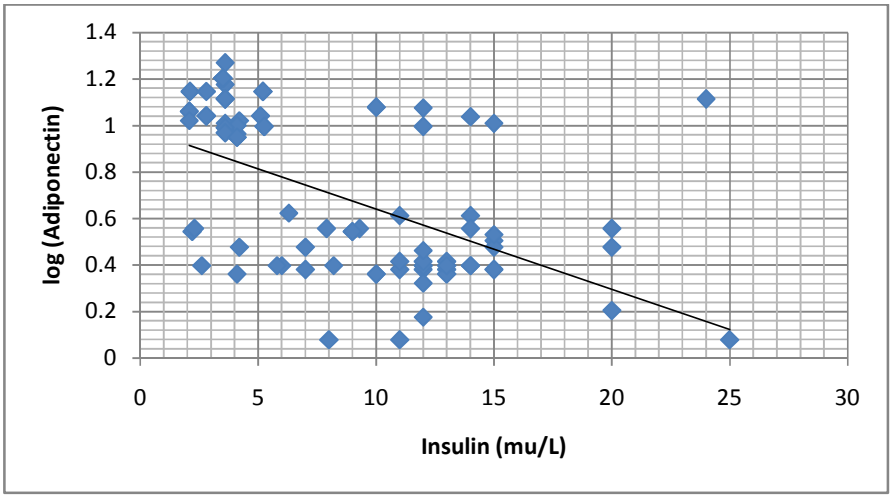

Fig. 1. Correlation between serum levels of adiponectin and insulin

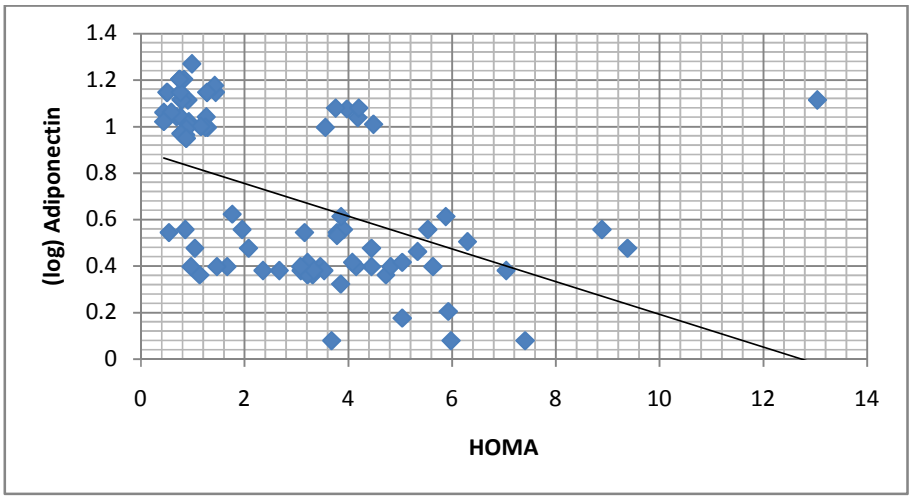

Fig. 2. Correlation between serum levels of adiponectin and HOMA
HOMA-IR = Homeostasis model assessment – insulin resistance

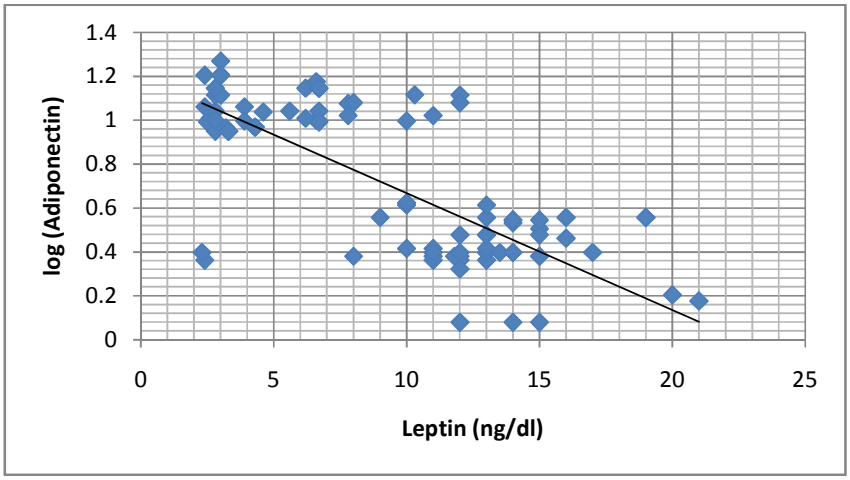

Fig. 3. Correlation between serum levels of adiponectin and leptin

Table 1. Anthropometric and clinical characteristics of NAFLD patients and control subjects

Parameters	NAFLD mean±SD	Control mean±SD	P value
M/F	30/20	15/25	NS
Age (year)	13.0±2.6	9.05±3.2	NS
BMI (Kg/m²)	34.6±5.5	25.8±4.6	<0.0001

BMI = Body mass index

Table 2. Biochemical parameters of NAFLD and control subjects

Parameters	NAFLD (N:50) mean± SD	Control (N:40) mean± SD	P value
Fasting glucose(mg/dl)	143±32.9	101±15.4	<0.0001
fasting insulin(mU/l)	11.4±4.9	4.7± 3.1	<0.0001
T. cholesterol(mg/dl)	170 ±29	164 ±29	NS
HDL cholesterol(mg/dl)	40±9.6	43 ± 8.0	NS
LDL cholesterol(mg/dl	130±27.5	108±11.2	<0.0001
Triglycerides (mg/dl)	192±37	156±31	<0.0001
HOMA	4.13±2.29	1.21±0.97	<0.0001
T. Bilirubin (mg/dl)	1.35±0.7	0.73±0.2	<0.0001
D. Biliurbin (mg/dl)	0.31±0.19	0.14±0.05	<0.0001
AST (U/l)	58.5±22.4	29.6±8.9	<0.0001
ALT (U/l)	63.3±30.4	27.8±6.1	<0.0001
GGT(U/l)	54.1±21.0	34.9±9.7	<0.0001
Albumin g/dl	3.6±0.5	3.9±0.2	<0.0001
Leptin(ng/dl)	13.1±3.2	4.8±2.3	<0.0001
Adiponectin(ng/dl)	3.23±2.5	11.0±2.95	<0.0001
CRP	18.7±13.2	4.99±3.2	<0.0001
Creatinine (mg/dl)	0.79±0.35	0.98±0.19	<0.0001
Alkaline phosphatase (U/l)	215±56.5	188±42.5	<0.05

*HOMA-IR = Homeostasis model assessment – insulin resistance, AST = aspartate transaminase, ALT = alanine transaminase, GGT: gamma glutamyltranspeptidase , CRP: C reactive protein . All values are represented as Mean ± Standard Deviation, *= There is a significant difference between groups by using independent t-test at p<0.05*

In our study, NAFLD prevalence appears to increase with age, with a mean age at 13 years and with a male-to-female ratio 3:2 and these results are in agreement with data presented by [13,14] and who suggested that NAFLD prevalence increases with age, with a mean age

at diagnosis between 11 and 13 years. This tendency is likely explained by adolescent hormonal changes, which result in an increase in serum insulin levels and fat accumulation in the liver [15-17]. Alisi and his associates (3) found that, according to epidemiological data, NAFLD in children increase in male than female in ratio 2:1.

We confirmed lower adiponectin level in children with NAFLD than in controls and these results are in agreement with data presented by Pagano et al. [18], Louthan et al. [19], Zou et al. [20], Burgert et al. [21] and Lebensztein et al. [22]. We also found no differences in the leptin levels between the group of children with NAFLD and controls and these results are consistent with the study of Mandato et al. [23]. Many authors found correlation between BMI and serum leptin [22-26] which is consistent with our findings. Hypoadiponectinemia was shown to be responsible for the accumulation of hepatic fat as well as the development of insulin resistance [27]. This finding is consistent with our study because we also confirmed a negative correlation between adiponectin and insulin resistance (HOMA-IR). Insulin resistance is common in obese children and it plays a major role in the pathogenesis of NAFLD [28]. It may play a role not only in the development of steatosis but also in the development of liver fibrosis by increasing hepatic fatty acid beta-oxidation stress [29]. We also found a negative correlation between ALT, AST & GGT and adiponectin in children with NAFLD and these results are in agreement with Lopez-Bermejo et al. [30] who reported that adiponectin levels were significantly correlated with ALT, GGT and ALP; thus suggesting a wider role for adiponectin in the maintenance of liver integrity. Also Lebensztein et al. [22] and Miriam et al. [31] found a negative correlation of serum adiponectin and ALT activity, which is consistent with our finding. Lebensztein et al. [22] reported that studies performed on children have special value because children could be regarded as an ideal model for the study of natural history and pathogenesis of obesity related liver disease for the earlier stages of the disease, absence of major confounding factor of liver pathology such as alcohol consumption and other environmental influences often seen in adults.

5. CONCLUSION

Our study reported a lower plasma adiponectin in NAFLD children that is inversely correlated with insulin resistance (HOMA-IR), ALT, AST and GGT. This data supports a role for adiponectin in protection against liver injury and put adiponectin to be a suitable serum marker in predicting liver steatosis in children with NAFLD, but these findings need to be confirmed in larger studies. We recommend a large-scale screening in the high-risk population, especially among the overweight pediatric patients, should be considered, including measurement of serum transaminases, plasma adiponectin and liver ultrasound. It is crucial to treat this condition as soon as possible in order to avoid the progression to end stage liver disease.

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CONFLICT OF INTEREST

All The authors declare that they have no competing interests.

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