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

Amal Ahmed Mohamed¹, Maha Abd El Ghany², Hassan Shalaby³, Dalal M. El-Melligy⁴, Khadega K. El Gohary⁵, Ayman Mohamed Abdel Aziz⁶ and Dalia A. El-Damasy⁷

¹Department of Biochemistry, National Hepatology and Tropical Medicine Research Institute, Egypt. ²Department of Pediatrics, El Sahel Teaching Hospital, Egypt. ³Department of Internal Medicine, Misr University for Science and Technology, Egypt. ⁴Department of Microbiology and Immunology, Ahmad Maher Teaching Hospital, Egypt. ⁵Department of Biochemistry, El Sahel Teaching Hospital, Egypt. ⁶Department of Hepatology and Gastroenterology, Theodor Bilharz Research Institute, Egypt. ⁷Department of Microbiology and Immunology, Egyptian Russian University, Egypt.

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.

Article Information

DOI: 10.9734/IJBcRR/2015/13737

(1) Chunying Li, Department of Biochemistry and Molecular Biology Wayne State University School of Medicine, Detroit, USA.

(1) Anonymous, Federal University of Triangulo Mineiro, Brazil.

(2) Anonymous, King Abdalaziz University, Saudi Arabia.

(3) Anonymous, Fukuoka University, Japan.

Complete Peer review History: http://www.sciencedomain.org/review-history.php?id=845&iid=3&aid=7409

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,
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 adipokine 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]. Adipokines, 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 CMV IgG 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):

\[ \text{HOMA-IR} = \frac{\text{fasting serum glucose (mg/dl)} \times \text{fasting insulin (\( \mu U/ml \))}}{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 ($r=0.445$, $P<0.0001$), with leptin ($r=0.725$, $P<0.0001$), with ALT ($r=0.534$, $P<0.0001$), with AST ($r=0.554$, $P<0.0001$), 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 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
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 liver fibrosis 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.

6. FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

All The authors declare that they have no competing interests.

REFERENCES

1. Barshop NJ, Sirlin CB, Schwimmer JB, Lavine JE. Review article. Epidemiology, pathogenesis and potential treatments of paediatric non alcoholic fatty liver disease. Aliment Pharmacol Ther. 2008;28:13-24.

2. Patton HM, Sirlin C, Behling C, Middleton MS, Schwimmer JB, Lavine JE. Pediatric non alcoholic fatty liver disease: A critical appraisal of current data and implications for future research. J Pediatr Gastroenterol Nutr. 2006;43:13-27.

3. Alisi A, Manco M, Vania A, Nobili V. Pediatric non alcoholic fatty liver disease in 2009. J Pediatr. 2009;155:469-474.

4. Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric non alcoholic fatty liver disease. Circulation. 2008;118:227-283.

5. Nobili V, Svegliati-Baroni G, Alisi A, Miele L, Valenti L, Vairo PA. 360-degree overview of pediatric NAFLD: Recent insights. J Hepatol. 2012;58(6):1218-1229.

6. Samuel VT, Liu ZX, Qu X, Elder BD, Bliz S, Befroy D, Romanelli AJ, Shulman GI. Mechanism of hepatic insulin resistance in

Mohamed et al.; IJBcRR, 5(4): 225-232, 2015; Article no.IJBcRR.2015.026

230
non-alcoholic fatty liver disease. J Biol Chem. 2004;279:32345-32353.

7. Yesim O, Ozlem BS. Fatty liver in childhood. World Journal of Hepatology. 2014;6(1):33-40.

8. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257:79-83.

9. Berardis S, Sokal E. Pediatric non-alcoholic fatty liver disease: An increasing public health issue. Eur J Pediatr. 2014;173:131-139.

10. Ozturk Y, Soylu OB. Fatty liver in childhood World J Hepatol. 2014;6(1):33-40.

11. Sanyal AJ AGA. Technical review on nonalcoholic fatty liver disease. Gastroenterology. 2002;123:1705-1725.

12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

13. Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009;136:1585-1592.

14. Widhalm K, Ghods E. Nonalcoholic fatty liver disease: A challenge for pediatrics. Int J Obes (Lond). 2010;34:1451-1467.

15. Roberts EA. Pediatric non alcoholic fatty liver disease (NAFLD): A “growing” problem? J Hepatol. 2007;46:1133-1142.

16. Romeo S, Huang-Doran I, Baroni MG, Kotronen A. Unravelling the pathogenesis of fatty liver disease: Palatin like phospholipase domain-containing 3 protein. Curr Opin Lipidol. 2010;21:247-252.

17. Deboer MD. Ethnicity, obesity and the metabolic syndrome: Implications on assessing risk and targeting intervention. Expert Rev Endocrinol Metab. 2011;6:279-289.

18. Pagano C, Soardo G, Esposito W, Fallo F, Basan L, Donnini D, et al. Plasma adiponectin is decreased in non alcholic fatty liver disease. European Journal of Endocrinology. 2005;152:113-118.

19. Louthan MV, Barve S, McClain CJ, Joshi-Brave S. Decreased serum adiponectin: An early event in pediatric non alcoholic fatty liver disease. J Pediatr. 2005;147(6):835-8.

20. Zou CC, Liang L, Hong F, Fu JF, Zhao ZY. Serum adiponectin, resistin levels and non-alcoholic fatty liver disease in obese children. Endocr J. 2005;52(5):519-24.

21. Burgert TS, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: Associations with insulin resistance, adiponectin and visceral fat. J Clin Endocrinol Metab. 2006;91(11):4287-94.

22. Lebenszttein DM, Wójtkowska M, Skiba E, Werpachowska L, Tobolczyk J, Kaczmarsi M. Serum concentration of adiponectin, leptin and resistin in obese children with non-alcoholic fatty liver disease. Advances in Medical Sciences. 2009;54(2):177-182.

23. Mandato C, Lucariello S, Licenziati MR, Franzese A, Spagnuolo MI, Ficarelli R, et al. Metabolic, hormonal, oxidative and inflammatory factors in pediatric obesity – related liver disease. J Pediatr. 2005;147(1):62-6.

24. Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M, et al. Serum leptin levels in patients with non alcoholic steatohepatitis. Am J Gastroenterol. 2000;95(12):3584-9.

25. Nakamuta M, Tada S, Uchimura K, Enjoji M, Kinukawa N, Iwamoto H, et al. Serum leptin levels in patients with nonalcoholic chronic liver disease. Hepatogastroenterology. 2001;48(38):527-32.

26. Steinberger J, Steffen L, Jacobs DR, Morant A, Hong CP, Sinaiko AR. Relation of leptin to insulin resistance syndrome in children. Obes Res. 2003;11(9):1124-30.

27. Musso G, Gambino R, Durazzo M, Biroli G, Carello M, Fagà E, et al. Adipokines in NASH: Postprandial lipid metabolism as a link between adiponectin and liver disease. Hepatology. 2005;42(5):1175–1183.

28. Ciba I, Widhalm K. The association between non-alcoholic fatty liver disease and insulin resistance in 20 obese children and adolescents. Acta Paediatr. 2007;96(1):109-12.

29. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et
al. Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183–1192.

30. Lopez-Bermejo A, Botes P, Funahashi T, Delgado E, Kihara S, Ricart W, Manual FJ. Adiponectin, hepatocellular dysfunction and insulin sensitivity. Clinical Endocrinology. 2004;60:256-263.

31. Miriam VL, Shirish B, Craig J, Swati JB. Decreased Serum Adiponectin: An early event in pediatric non-alcoholic fatty liver disease. The Journal of Pediatrics. 2005;147:835-838.

© 2015 Mohamed et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=845&id=3&aid=7409