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Original Article

Fibroblast Growth Factor-2 and Tumor Necrosis Factor-Stimulated Gene-6: New Biomarkers for Non-Alcoholic Fatty Liver Disease in Obese or Overweight Children?

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ABSTRACT

Aim: Pediatric non-alcoholic fatty liver disease (NAFLD) is the leading chronic liver disease in children, closely linked to obesity. While liver biopsy remains the gold standard for diagnosis, there is an urgent need for non-invasive biomarkers. This study evaluated the potential diagnostic value of fibroblast growth factor-2 (FGF-2) and tumor necrosis factor-stimulated gene-6 (TSG-6) levels in pediatric NAFLD.

Materials and Methods: This cross-sectional study included 38 children diagnosed with NAFLD via ultrasonography and 26 healthy controls. The patient group consisted of obese or overweight children with NAFLD attending a pediatric gastroenterology clinic. Healthy controls were age-matched, non-obese children without chronic diseases or active infections. Serum FGF-2 and TSG-6 levels were measured in both groups.

Results: Among the 38 NAFLD patients (16 girls, 22 boys) and 26 controls (10 girls, 16 boys), the median FGF-2 level was significantly lower in the patient group (107.50 pg/mL, range: 25.90-533.80) compared to the controls (183.05 pg/mL, range: 50.90-709.80) (p=0.033). The median TSG-6 level was 3,564.60 pg/mL (range: 2,497.50-4,366) in the patient group and 3,504.15 pg/mL (range: 2,370.70-4,366) in the control group, with no statistically significant difference (p=0.199).

Conclusion: Lower FGF-2 levels may play a crucial role in NAFLD pathophysiology and serve as a potential biomarker for diagnosis. Further research is needed in order to validate these findings and to explore their clinical implications.

Keywords: Children, FGF-2, non-alcoholic fatty liver disease, TSG-6, obese, overweight

Introduction

Pediatric non-alcoholic fatty liver disease (NAFLD) stands as the most prevalent chronic liver condition in children, with its incidence steadily increasing alongside rising rates of overweight and obesity (1,2). The World Health Organization identifies being overweight or obese as the fifth most significant risk factor for global mortality (3). This escalating public health crisis is largely attributable to sedentary lifestyles. NAFLD is not merely a liver disorder; it is a multisystem disease which can impact various organs, leading to substantial morbidity and mortality (1,2). The term NAFLD encompasses

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a wide spectrum of conditions, ranging from isolated hepatic steatosis-characterized by fat accumulation without inflammation-to non-alcoholic steatohepatitis, which has the potential to progress to end-stage liver disease (1). Alarmingly, the prevalence of NAFLD is rising exponentially, paralleling the increasing rates of obesity and type 2 diabetes mellitus in both children and adults (4,5). Although the precise prevalence of NAFLD remains unclear, it is estimated to affect approximately 34% of obese children and around 10% of the general pediatric population (5). The pathophysiology of NAFLD stems from intricate hepatocellular metabolic dysfunctions which disrupt insulin action, impair fat metabolism, and free fatty acid processing, and subsequently lead to oxidant-mediated hepatocyte damage (6).

Fibroblast growth factor-2 (FGF-2) has demonstrated notable antifibrotic effects and the ability to promote tissue regeneration, particularly in the context of fibrotic diseases (7,8). As a potent hepatotropic mitogen, FGF-2 plays a crucial role in hepatocyte function. Research indicates that FGF-2 stimulates the regeneration of the extracellular matrix following liver injury and regulates hepatocyte proliferation and migration in vitro (9,10). Additionally, FGF-2 has been shown to exert anti-inflammatory effects by modulating cluster of differentiation (CD) 40 expression and the CD40-CD40L signaling pathway (11).

Furthermore, previous studies have highlighted the interaction between tumor necrosis factor-stimulated gene-6 (TSG-6) and the CD44 receptor on hepatic stellate cells (HSC), positioning TSG-6-based therapy as a promising target for treating liver fibrosis. TSG-6 is also recognized for its anti-inflammatory and tissue-protective properties (12,13).

While liver biopsy remains the gold standard for diagnosing NAFLD, its poor acceptance among patients underscores the urgent need for reliable, accurate, and non-invasive or minimally invasive biomarkers. In this study, we explored the hypothesis that levels of FGF-2 and TSG-6 could effectively differentiate between obese or overweight children with NAFLD and healthy controls.

Materials and Methods

This study included both a patient group and an age- and sex-matched healthy control group. The patient group comprised obese or overweight children under the age of 18 diagnosed with NAFLD through ultrasonographic examination at a pediatric gastroenterology, hepatology, and nutrition outpatient clinic between 2020 and 2022.

Anthropometric measurements were taken with children wearing light clothing and without shoes. At the time of admission, height, weight, and waist circumference measurements were recorded for the patient group. Body mass index (BMI) was calculated by dividing the patient's weight in kilograms by the square of their height in meters. A BMI of 25 to 29.9 is classified as overweight, while a BMI over 30 is categorized as obese. Patients with a history of medication use due to any chronic disease were excluded from this study. The control group consisted of children under age 18 with normal BMI according to their age and sex who presented to the pediatric outpatient clinic and who did not have any chronic diseases, active infections, or histories of chronic medication use. Liver steatosis is categorized into three grades based on ultrasonographic findings: Grade 1 (mild hepatic steatosis), Grade 2 (moderate hepatic steatosis), and Grade 3 (severe hepatic steatosis). Written informed consent was obtained from the parents of all participants in both groups, and ethical approval was obtained from the Cumhuriyet University Clinical Research Ethics Committee decision no.: 2022-05/03, dated: 31.05.2022).

Sample Collection

Fasting blood samples were collected at 9:00 a.m. into serum tubes and K3- ethylenediaminetetraacetic acid (K3-EDTA) tubes (Becton Dickinson, UK) from all patients and healthy controls. Patient samples were obtained upon admission. Serum tube samples were allowed to clot before centrifugation. After centrifugation at 4 °C for 15 minutes at 3,500 rpm, the serum was aliquoted and immediately frozen at -80 °C. K3-EDTA tubes were analyzed promptly.

Biochemical Analyses

The quantitative sandwich enzyme-linked immunosorbent assay technique was used for the determination of serum FGF-2 (Wuhan USCN Business Co., Ltd, China) and TSG-6 (Cloud Clone Corp.). Complete blood count, glucose, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, total bilirubin, direct bilirubin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total cholesterol, triglyceride, albumin, ions, C-reactive protein (CRP) and erythrocyte sedimentation rate values were evaluated.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences 22.0 computer software. The normality of distribution of numerical variables was evaluated. Numerical

data were compared between the groups using the Mann-Whitney U test (non-normally distributed subjects) and the sample t-test (normally distributed subjects). Descriptive statistics were used to report on the demographical and clinical data from the NAFLD patients and the healthy controls. He data are expressed as medians, ranges, means and standard deviations (SD). Differences in continuous variables between the patients from each subgroup, and between the patients and the healthy controls were analyzed using the Mann-Whitney U test. The Kruskal-Wallis test was used for non-parametric numerical variables of multiple groups. We used Spearman's rank difference correlation to examine the correlation between TSG-6 and FGF-2 and acute phase reactants, and complete blood count parameters. A p-value less than or equal to 0.05 was considered statistically significant.

Results

A total of 38 children [16 girls (42%) and 22 boys (58%)] diagnosed with NAFLD and 26 healthy controls (10 girls and 16 boys) were included in this study. The mean age of the patient group was 12.55±3.04 years, while the mean age of the control group was 10.73±4.27 years.

In the patient group, the mean weight was 76.72±19.79 kg, the mean height was 157.75±15.67 cm, the mean BMI was 30.27±3.71, and the mean waist circumference was 99.17±10.05 cm. Additionally, the mean weight SD was 2.97±0.98, the height SD was 1.03±1.20, and the BMI SD was 2.62±0.65.

Laboratory data for both the patient and control groups are summarized in Table I. Although the absolute absolute neutrophil count/absolute lymphocyte count ratio (1.63±1.04 vs. 1.28±0.80, respectively) and white blood cell count (7,989.7± 2,277.66 vs. 7,180.38±1,499.10, respectively) were higher in the patient group, no statistically significant differences were observed between the two groups.

When comparing the two groups in terms of TSG-6 levels, the median TSG-6 level in the patient group was 3,564.60 pg/mL (range: 2,497.50-4,366), while the median TSG-6 level in the control group was 3,504.15 pg/mL (range: 2,370.70-4,366). This difference between the two groups was not statistically significant (p=0.199).

In contrast, when evaluating FGF-2 levels, the median FGF-2 level in the patient group was 107.50 pg/mL (range: 25.90-533.80), compared to a median of 183.05 pg/mL (range: 50.90-709.80) in the control group. This difference was statistically significant (p=0.033).

Radiologically, twenty-four patients exhibited Grade 1 hepatic steatosis, twelve had Grade 2, and two presented with Grade 3. Upon evaluating TSG-6 and FGF-2 levels across these hepatic steatosis grades, no statistically significant differences were found (p=0.777 for TSG-6 and p=0.624 for FGF-2).

Discussion

The prevalence of NAFLD among children is increasing alarmingly, presenting a significant health concern associated

Table I. Laboratory findings of the patient group and the heathy control group

	NAFLD n=38	**Heathy control n=26**	**p-value**
White blood cell (103/uL), mean ± SD	7,989.47±2,277.66	7,180.38±1,499.10	NS
Absolute neutrophil count/ Absolute lymphocyte count, mean ± SD	1.63±1.04	1.28±0.80	NS
TSG-6 (pg/mL), median (minimum-maximum)	3,564.60 (2,497.50-4,366)	3,504.15 (2,370.70-4,366)	NS
FGF-2 (pg/mL), median (minimum-maximum)	107.50 (25.90-533.80)	183.05 (50.90-709.80)	**0.033**
AST (U/L), median (minimum-maximum)	29 (16-105)	27 (9-35)	NS
ALT (U/L), median (minimum-maximum)	24 (8-244)	22 (11-33)	NS
Triglyceride, mean ± SD	110.52±44.03	105.33±24.30	NS
Total cholesterol, mean ± SD	161.45±39.12	124.15±22.34	NS
LDL-cholesterol, mean ± SD	110.70±30.17	105.51±33.21	NS
HDL-cholesterol, mean ± SD	43.16±8.26	48.45±19.28	NS
ESR (mm/h), mean ± SD	10.54±6.99	12.43±3.12	NS
CRP (mg/L), median (minimum-maximum)	0.37 (0.10-13.40)	0.32 (0.14-4.40)	NS

NAFLD: Non-alcoholic fatty liver disease, FGF-2: Fibroblast growth factor-2, TSG-6: Tumor necrosis factor-stimulated gene-6, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, NS: Not significant

with high morbidity and mortality rates. Despite this, there is a relative scarcity of studies focusing on NAFLD in pediatric populations when compared to adults. Our study is pioneering in evaluating FGF-2 and TSG-6 levels in overweight or obese children diagnosed with NAFLD. A thorough literature review revealed no existing studies investigating TSG-6 and FGF-2 levels specifically in this demographic.

FGFs play a crucial role in regulating HSC differentiation and liver fibrosis, along with exhibiting anti-inflammatory effects. FGF-2, a significant member of the FGF family, is pivotal in modulating HSC function, injury repair, and tissue regeneration. Active HSCs are primary drivers of extracellular matrix deposition in liver fibrosis. FGF-2 has demonstrated anti-fibrotic properties and the ability to promote tissue regeneration in fibrotic diseases, including liver fibrosis (7,14,15). It primarily interacts with FGF receptor-1, which is significantly overexpressed in activated HSCs, thereby inhibiting their activation. Kurniawan et al. (7) proposed a promising therapeutic approach utilizing FGF-2 for the treatment of liver fibrosis.

In obesity, adipocytes secrete pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, which stimulate hepatic production of acute phase proteins, including CRP, and alter the immune response (16). Additionally, serum adiponectin levels, which are known for their anti-inflammatory effects, are diminished. The increase in reactive oxygen species and reduction in antioxidant substances further exacerbate this negative shift in the immune response, tipping the balance toward inflammation and subsequently decreasing growth factor production (17).

Our findings indicate that FGF-2 levels in the patient group were statistically significantly lower than those in the control group. This suggests a potential dysregulation of FGF-2 synthesis in obese or overweight pediatric patients with NAFLD. Insufficient FGF-2 expression may contribute to the development of NAFLD, positioning FGF-2 injection as a potential therapeutic intervention. The observed low FGF-2 levels in obese patients may reflect a shift toward pro-inflammatory processes.

TSG-6, a cytokine released by human mesenchymal stem/stromal cells (MSC), possesses anti-inflammatory and hepatoprotective effects (12,13). TNF-α and IL-1 activate the transcription of the TSG-6 gene in human fibroblasts (18). TSG-6 is produced in response to various inflammatory stimuli and exhibits anti-inflammatory effects through multiple mechanisms (19,20). Recent studies have identified TSG-6 as a crucial factor in inducing the immunoregulatory effects of MSC and as a promising biomarker for their therapeutic effects (21). Wang et al. (13) demonstrated the therapeutic benefits of TSG-6 injections in mice with liver fibrosis, while Miyaji et al. (22) showcased similar effects in rats with liver damage.

In our study, we did not find a statistically significant difference in TSG-6 levels between obese or overweight children with NAFLD and the control group. Importantly, none of our patients presented with end-stage liver disease. The absence of significant differences in TSG-6 levels may be attributed to the fact that NAFLD had not progressed to an advanced stage in our patient cohort.

Study Limitations

Our study does have limitations, notably the small sample size and the absence of liver biopsy for definitive diagnosis. Liver biopsy is invasive and carries risks such as sampling errors and complications (e.g., pain, bleeding, pneumothorax). One of the limitations of our study is the low number of patients in the liver steatosis groups. Another limitation of this study was the significantly smaller number of participants in the healthy group compared to the patient group. This situation may have had a negative impact on statistical power and so may reduce the validity and reliability of the results. Smaller sample sizes can make the findings more susceptible to random fluctuations, leading to questions about how representative the results are for the general population. Therefore, the limited number of individuals in the control group may restrict the generalizability of this study's findings.

Conclusion

In conclusion, our results suggest that low FGF-2 levels may play a role in the pathophysiology of NAFLD and could be beneficial for diagnostic purposes. However, further multicenter studies with larger patient cohorts are warranted in order to support these findings.

Ethics

Ethics Committee Approval: This study was approved by the Cumhuriyet University Clinical Research Ethics Committee (decision no.: 2022-05/03, dated: 31.05.2022).

Informed Consent: Written informed consent was obtained from the parents of all participants in both groups.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: E.K.T., Concept: E.K.T., K.D., Design: E.K.T., K.D., Data Collection or Processing: E.K.T., K.D., Analysis or Interpretation: E.K.T., Literature Search: E.K.T., K.D., Writing: E.K.T., K.D.

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