The role of insulin - like growth factor - 1 on steatohepatitis

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Abstract

***Purpose* : Recent studies have revealed that growth hormone and STAT5 were related to hepatosteatosis in mice. Loss of signal transducer and activator of transcription factor-5 leads to hepatosteatosis and impaired liver regeneration. We aimed to investigate the role of IGF-1 in steatosis with normal (SNLFT) and disturbed liver function tests (SDLFT) in humans.**

***Method* : We included 272 NAFLD patients and 110 age, sex and body mass index (BMI)-matched healty controls. We measured routine blood biochemistry and complete blood count, IGF-1, insulin, c-peptide, ferritin, hsCRP, ESR and HOMA-IR. We subdivided NAFLD patients into SNLFT and SDLFT subgroups.**

***Results* : Age, sex and BMI were similar between NAFLD and controls. IGF-1 levels were significantly lower in NAFLD patients (120,6±48,2) than controls (148,9±53,8), (p<0,0001). IGF-1 levels were also lower in SDLFT subgroup (93,4±27,8) than SNLFT subgroup (123,1±49,0), (p:0,032). Waist circumference, fasting blood glucose, HbA1c, uric acid, hsCRP, AST, ALT, GGT, WBC, hemoglobin, hematocrit, ferritin, insulin, c-peptid and HOMA-IR measurements were significantly higher in NAFLD patients than controls (for all values: p<0,0001).Cholesterol (p:0,026), triglycerides (p<0,0001), ESR (p:0,006) were significantly higher in NAFLD patients than controls. HDL-chelesterol levels were significantly lower (p:0,002) in NAFLD patients than controls.**

***Conclusion* : This study supported previous findings of experimental studies in that, IGF-1 levels were lower in SNLFT and SDLFT. Growth hormone-IGF-1 system may be involved in the pathogenesis of NAFLD.** (Acta gastroenterol. belg., **2017**, 80, **21-24**).

Key words : IGF-1, non-alcoholic fatty liver disease.

Introduction

Growth hormone (GH) has important influence on carbohydrate, lipid and protein metabolisms. GH antagonizes insulin action on carbohydrate metabolism, stimulates lipolysis and increases circulating free fatty acids (FFA) levels. GH stimulates protein synthesis, increases lean mass and reduces fat mass (1). GH accomplishes most of its effects by insulin-like growth factor-1 (IGF-1), which is secreted by the liver. The liver maintains the synthesis IGF-I and its binding proteins, with the stimulation of GH. Chronic liver diseases are associated with GH resistance and low growth hormone binding protein (GHBP) and IGF-1 levels (2). Several studies suggest that there is a strong association between the GH/IGF-I axis and liver steatosis. It has been hypothesized that GH may have direct effect on hepatic lipid accumulation, additionally inhibition of endogenous GH signalling might induce liver steatosis (3). Furtheremore, NAFLD was more common in hypopituitary patients compared with controls and, was associated with severe GH deficiency (4).

In previous studies, the role of IGF-1 in steatosis with normal (SNLFT) and disturbed liver function tests (SDLFT) was not studied seperately. In this study, we aimed to investigate the influence of IGF-1 on NAFLD and its subgroups such as SNLFT and SDLFT.

Method

Population

We included 382 consecutive subjects referred to our endocrinology and gastroenterology outpatient clinics for suspected NAFLD between November 2010 and March 2011 in the study. The patients with and without NAFLD comprised NAFLD and control groups, respectively. Inclusion criteria were: no history of excessive alcohol consumption defined as an average consumption of alcohol < 30 g/day in men and < 20 g/day in women; negative hepatitis B surface antigen and antibody to hepatitis C virus; absence of history and findings consistent with cirrhosis. Other chronic liver diseases such as autoimmune hepatitis, Wilson's disease and hemochromatosis were excluded by the measurements of autoimmune markers of hepatitis, serum ceruloplasmin and ferritin levels respectively. We excluded overt diabetic patients in order to eliminate the effect of diabetes on IGF-1. Anthropometric measurements were recorded for each subject. The study protocol is in accordance with the Declaration of Helsinki and was approved by local ethics committee. Each patient gave his or her written informed consent, and the study was conducted according to the rules of good clinical practice.

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Biochemical analysis

Fasting serum total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, (Lot No: B302, Konelab), g-glutamyltransferase (GGT) (Lot No: C331, Konelab), alanine aminotransferase (ALT) (Lot No: C239, Konelab) and aspartate aminotransferase (AST) (Lot No: C372, Konelab) concentrations were measured enzymatically with an automatic analyzer (Konelab 60İ, Thermo Scientific, Filland). Total cholesterol (Lot No: B540, Konelab) and triglycerides (Lot No: C186, Konelab) were measured with enzymatic colorimetric tests, low-density lipoprotein-cholesterol (Lot No: C435, Konelab) and HDL-C (Lot No: C136, Konelab) were measured with the homogeneous enzymatic colorimetric test. The serum creatinine was measured with the alkaline picrate (Jaffe) method (Lot No: C092, Konelab). Fasting serum insulin and C peptid levels were measured by Liaison Immunoluminometric Assay (DiaSorin Inc.Stillwater, MN, USA). HbA1c analyses performed by an automated Tosoh G7 HbA1c Analyzer (Tosoh Corporation, Tokyo, Japan). Serum IGF-1 concentration was measured by chemiluminescence method (Immulite® 1000 Siemens Healthcare diagnostics IL, USA)

NAFLD evaluation

Liver ultrasonography (US) scanning was performed to assess the degree of steatosis. All US were performed by the same operator who was unaware of the aims of the study and blinded to laboratory values using an General Electric Logic 5 apparatus equipped with a convex 3,5 MHz probe. We further subdivided NAFLD patients into SDLFT and SNLFT, according to presence or absence of abnormalities in blood liver function tests, respectively.

Statistics

Statistical Package for Social Sciences (SPSS) version of 15.0 was used for analysis. Distribution of data was assessed by using one-sample Kolmogorov-Smirnov test. Continuous variables were expressed as mean±SD, categorical variables were expressed as percentage. For comparison of categorical variables or percentages we used Fisher's exact and chi-square tests. Differences between numeric variables were tested with Student's t-test or Mann-Whitney U-test. Correlation was tested with Spearman's rank order or Pearson correlation coefficient. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors for the presence of SNLFT. Hosmer-Lemeshow goodness of fit statistics were used to assess model fit. A p value below 0.05 was considered as statistically significant.

Results

Age, sex and BMI were similar between NAFLD and controls. IGF-1 levels were significantly lower in NAFLD patients (120,6±48,2) than controls (148,9 ±53,8), (p<0,0001). IGF-1 levels were also significantly lower in SDLFT subgroup (93,4±27,8) than SNLFT subgroup (123,1±49,0), (p:0,032) (Figure 1). Waist circumference, fasting blood glucose, HbA1c, uric acid, hsCRP, AST, ALT, GGT, WBC, hemoglobin, hematocrit, ferritin, insulin, c-peptid and HOMA-IR measurements were significantly higher in NAFLD patients than controls (for all values: p<0,0001). Total cholesterol (p:0,026), triglycerides (p<0,0001), ESR (p:0,006) were significantly higher in NAFLD patients than controls. HDL-cholesterol levels were significantly lower (p:0,002) in NAFLD patients than controls (Table 1).

IGF-1 levels were negatively correlated with age (r:-0,312, p<0,0001), BMI (r:-0,217, p:0,042), waist circumference (r:-0,307, p<0,0001), fasting blood glucose (r:-0,249, p:0,001). The logistic regression analysis showed that IGF-1 (OR: 0.990, %95CI: 0.983-0.997, p:0.004) and insulin (OR: 1.177, %95CI: 1.095-1.266, p<0,0001) levels can significantly predict the presence of NAFLD. The number of patients classified as metabolic syndome in NAFLD group (n: 118/272) were higher than controls (n: 24/110)(p<0,0001). The patients with metabolic syndrome had lower IGF-1 levels, than patients without metabolic syndrome respectively (median:204, minimum:4,15-maximum:220 vs median: 139,4, minimum: 49,43-maximum: 313,4 ng/ml).

Discussion

In this study, we found that IGF-1 levels were significantly lower in NAFLD group than controls. IGF-1 levels were even lower in the SDLFT subgroup than the SNLFT subgroup. IGF-1 and insulin were found to be strong predictors of NAFLD in multivariate analysis.

In previous studies IGF-1 levels were dependent on the degree of the glucose control, with near normal IGF-I

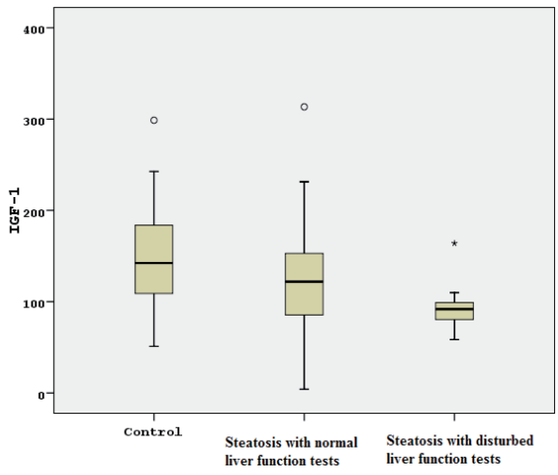

Fig. 1. — The distribution of IGF-1 levels among SDLFT, SNLFT subgroups and controls.

Table 1. — **Demographic features and biochemistry test results of NAFLD and control subjects**

	NAFLD (n:272)	**CONTROL (n:110)**	***p***
Age (years)	46,3±10,9	44,2±11,0	NS
BMI (kg/m^2)	31,9±5,1	30,3±5,3	NS
Waist circumference (cm)	103,0±12,2	95,7±11,5	<0,0001
HOMA-IR	3,75±3,14	2,44±2,15	<0,0001
HbA1c (%)	5,97±0,58	5,58±0,35	<0,0001
Fasting Blood Glucose (mg/dl)	100,1±19,2	92,8±15,6	<0,0001
BUN (mg/dl)	26,9±8,3	25,8±7,6	NS
Creatinine (mg/dl)	0,87±0,17	0,83±0,16	NS
Uric acid (mg/dl)	5,4±1,2	4,6±1,2	<0,0001
ALT (IU/L)	39,7±37,2	20,8±8,8	<0,0001
AST (IU/L)	30,2±17,8	23,4±20,2	<0,0001
GGT (IU/L)	37,0±29,6	25,8±26,8	<0,0001
ALP (IU/L)	80,1±36,7	75,3±39,5	0,012
hsCRP (mg/dl)	1,93±4,17	0,93±1,81	<0,0001
Ferritin (ng/ml)	58,5±55,5	35,7±31,5	<0,0001
Insulin (μIU/mL)	14,6±9,3	10,5±8,1	<0,0001
C peptide (ng/ml)	3,26±1,60	2,45±1,15	<0,0001
Total cholesterol (mg/dl)	199,3±40,8	188,2±39,8	0,008
HDL-cholesterol (mg/dl)	44,5±11,7	49,3±14,6	0,001
LDL-cholesterol (mg/dl)	119,8±37,4	116,1±33,3	NS
Triglycerides (mg/dl)	172,0±106,3	125,0±68,9	<0,0001
ESR (mm/hour)	16,6±12,7	12,7±11,7	0,001
IGF-1 (ng/ml)	120,6±48,2	148,9±53,8	0,001
Metabolic syndrome (number of patients/population)	n: 118/272	n: 24/110	<0,0001

levels in well-controlled diabetes, whereas they tended to decrease in poorly controlled diabetes (5,6). Low IGF-1 levels were also found to be related to increased mortality in type 2 diabetic patients (7). Additionally, IGF-1 improves glucose sensitivity directly (8) and indirectly by decreasing GH secretion as a negative feedback mechanism (9). The somatotropic axis is also affected by severe obesity, and low IGF-1 concentrations have been demonstrated in obese subjects (10). In our study, we found significant negative correlation between IGF-1 and BMI, and fasting blood glucose levels in accordance with these previous studies.

Growth hormone-IGF-1 system has been implicated in the process of hepatosteatosis. It has been known that patients with pituitary failure were prone to obesity, impaired glucose tolerance, and dyslipidemia with subsequent development of hepatosteatosis and an in-creased prevalence of cirrhosis (11). In a retrospective ana-lysis of 44 patients with GH deficiency, the prevalence of NAFLD increased by 29% after the cessation of GH in adult patients with childhood onset GH deficiency (12). It has also been reported that GH replacement therapy reversed NASH in a case of adult growth hormone deficiency (AGHD) (13). Nishizawa et al. reported that the prevalence of NAFLD was significantly higher in 66 Japanese patients with AGHD compared to controls (77 vs. 12%, p< 0.001) (14). Aditionally, GH replacement therapy significantly reduced serum liver enzyme levels and improved the histological changes in the liver in patients with NASH. Meanwhile, liver-specific deletion of GH receptor in mice (GHRLD) with very low serum IGF-1 levels, resulted in severe steatosis (3). Liver regeneration was impaired in this model, indicating that GH also regulates the proliferation capacity of hepatocytes. These results imply that IGF-I, but not GH itself, prevents the development of NAFLD.

The specific deletion of IGF-1 in the liver results in insulin resistance (15), indicating that hepatic IGF-I regulates systemic insulin sensitivity. IGF-1 may reverse NAFLD by improving insulin resistance. IGF-I may also be improving oxidative stress in the liver, by regulating mitochondrial function. Hao et al have reported that IGF-I improves mitochondrial function (16). Perez et al. showed that IGF-I administration improved liver dysfunction and fibrosis in a rat cirrhosis model, and mitochondrial function in aging rats (17).

After these findings, several pilot studies were carried out to show the relation between SNLFT and GH in populations without GH deficiency. Ichikawa et al showed that, GH, IGF-I, and IGF binding protein-3 (IGFBP-3)

Table 2. — **Correlation between IGF-1 and measured parameters (WC: waist circumference, FBG: fasting blood glucose, ESR: erythrocyte sedimentation rate)**

IGF-1	Age	BMI	WC	FBG	hsCRP	AST	ALP	Albumin	Insulin	c-peptide	ESR
r	-0,312	-0,217	-0,307	-0,249	-0,158	-0,182	-0,224	-0,247	-0,144	-0,164	-0,260
p	<0,0001	0,042	<0,0001	0,001	0,033	0,012	0,001	0,001	0,048	0,027	<0,0001

were associated with hepatic steatosis and fibrosis in patients with NAFLD (18). Arturi et al showed that non-diabetic patients with NAFLD had significantly higher BMI, waist circumference, fasting insulin, triglycerides, insulin resistance, ALT, AST, GGT, and lower HDL-cholesterol as compared with the non-NAFLD group. IGF-I levels were significantly lower in the NAFLD group (19). In another study, NAFLD group had lower IGF-1 levels as compared with the non-NAFLD group (20). Fusco et al also found that patients with NAFLD had higher levels of GH-binding protein and IGFBP-3 levels, and lower GH peak and IGF-I levels as compared to controls (21). In addition to these previous studies, we further divided NAFLD patients into SDLFT and SNLFT subgroups, and found even lower IGF-1 levels in SDLFT subgroup.

The molecular mechanisms behind hepatosteatosis and lowered IGF-1 levels remain elusive. However there are some clues which point out liver-specific signal transducer and transcriptional activator 5 (STAT5). Mice with a STAT5 ablation developed steatosis, glucose intolerance, insulin resistance, late-onset obesity, and impaired liver regeneration. Recent studies with liver-specific STAT5-deficient mice suggest that elevated CD36, PPARγ, and PPARγ coactivator 1 alpha/beta (PGC1α/β), along with increased fatty acid synthesis, lipoprotein lipase, and VLDL receptor, are associated with hepatic steatosis in these mice (22). Furtheremore, deletion or silencing of IGF-1 receptor (IGF-1R) results in diminished GH-induced STAT5 phosphorylation, suggesting that the presence of IGF-1R may facilitate GH signaling (23).

In conclusion, in this study we found that IGF-1 levels are decreased in NAFLD patients, with the lowest levels in SDLFT subgroup. Further experimental studies are needed to reveal the molecular connection between IGF-1 levels and hepatosteatosis.

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