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A case–control study: Association between serum neuregulin 4 level and non-alcoholic fatty liver disease

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ABSTRACT

Background. Non-alcoholic fatty liver disease (NAFLD) is a great health burden. Neuregulin 4 (Nrg4) is a recently identified secret factor that may be associated with NAFLD.

Aim. To investigate the association between serum Nrg4 level and NAFLD by conducting a case–control study.

Method. A total of 174 subjects were included. 87 NAFLD subjects and 87 age- and sex-matched non-NAFLD controls were identified by hepatic ultrasound examination. Anthropometric and biochemical data were measured and recorded. Serum Nrg4 level was evaluated by using enzyme-linked immunosorbent assay. SPSS software was used for statistical analyses.

Results. Compared to the controls, subjects with NAFLD presented with reduced level of serum Nrg4 (0.40 (0.27, 0.55) vs. 0.50 (0.30, 0.81) ng/mL (median (interquartile range)), $P = 0.029$). By multivariate logistic regression analysis, reduced serum levels of Nrg4 were associated with higher NAFLD odds (OR = 0.251, 95% confidence interval = 0.081–0.779, $P = 0.017$). By dividing the distribution of serum Nrg4 level into quartiles, there was borderline statistical difference of NAFLD prevalence among the four groups ($P = 0.058$). There was no significant difference of serum Nrg4 levels in subjects according to the grades of fatty liver by ultrasound ($P = 0.080$). No statistical difference of serum Nrg4 level was observed between obese and non-obese subjects ($P = 0.932$).

Conclusion. Decreased serum Nrg4 level is prevalent in NAFLD subjects compared to non-NAFLD controls, and is an independent risk factor associated with NAFLD, indicating that Nrg4 might have a protective role in the development of NAFLD.

Abbreviations: AFP, Alpha-fetoprotein; AKP, Alkaline phosphatase; ALT, Alanine aminotransferase; ANOVA, One-way analysis of variance; AST, Aspartate aminotransferase; BAT, Brown adipose tissue; BMI, Body mass index; DBP, Diastolic blood pressure; ELISA, Enzyme-linked immunosorbent assay; GGT, Gamma-glutamyltransferase; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic fatty liver steatohepatitis; Nrg4, Neuregulin 4; SBP, Systolic blood pressure; VLDL, Very low-density lipoprotein.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases, affecting approximately 30% of the general population worldwide [1]. NAFLD covers a spectrum of liver diseases ranging from simple steatosis to steatohepatitis (NASH), to fibrosis and finally cirrhosis [2]. Currently, it is widely accepted that NAFLD is the hepatic manifestation of insulin resistance and metabolic syndrome [3].

Adipokines are emerging as predictors for the functional status of adipose tissue, and play a pivotal role in the regulation of metabolism, inflammation, and insulin sensitivity [4–6]. Literature has indicated that altered concentrations of circulating adipokines, e.g. leptin, adiponectin and irisin, might be associated with NAFLD [7–9].

Neuregulin 4 (Nrg4) is a member of the epidermal growth factor (EGF) family of extracellular ligands. As a novel endocrine factor, it acts as autocrine, paracrine or endocrine signal by releasing the EGF-like domain after photolytic cleavage [10]. Based on animal studies, Nrg4 is markedly enriched in brown adipose tissue (BAT), up-regulated in white fat tissue upon cold exposure, and highly expressed during brown adipocyte differentiation [11,12]. Recently, Wang *et al* have demonstrated that Nrg4 acts as an endocrine link between BAT and liver lipogenesis. According to the study by Wang *et al*, Nrg4 deficiency exacerbates hepatic steatosis and insulin resistance, and the expression of Nrg4 mRNA is lower in humans with impaired glucose tolerance [12].

Therefore, we hypothesized that Nrg4 plays a protective role in the development of NAFLD. In this study, we tried to explore the association between serum Nrg4 levels and NAFLD by conducting a case–control study. It is the first clinical investigation regarding to serum Nrg4 concentrations in human subjects with NAFLD, with the purpose of filling the gap in the research on Nrg4 and human NAFLD.

2. Methods

2.1. Study Design and Patients

NAFLD cases and non-NAFLD controls were selected from the patients admitted to the First Affiliated Hospital of Zhejiang University, School of Medicine between January 2015 and March 2015. Patients should be excluded if they had malignancy, severe cardiopulmonary disorders, renal dysfunction, acute or chronic inflammatory diseases, thyroid dysfunction and pregnancy. Excessive alcohol drinkers (defined as ethanol intake more than 140 g in men and 70 g in women per week) [13] were also excluded. Diabetes mellitus was not an exclusion criterion. The presence of fatty liver was evaluated by ultrasound. In the meantime, competing etiologies for steatosis, such as viral hepatitis, alcohol consumption (intake of ethanol more than 140 g in men and 70 g in women per week), drug-induced liver disease, autoimmune hepatitis, and hereditary disorders were excluded. We defined obese subjects whose body mass index (BMI) was more than 25 kg/m^2 and normal participants whose BMI was less than 25 kg/m^2 according to the World Health Organization.

The sample size was estimated according to the sample size formula. The significance level of α was set at 0.05, and the allowable error of δ was set at 0.01. The minimal sample size was seventy-three for cases and seventy-three for controls. In our study, the diagnosis of NAFLD was ascertained in eighty-seven patients. The other eighty-seven non-NAFLD subjects were included because they were matched with those NAFLD cases on age and gender. Therefore, a total of 174 participants were enrolled in this observational study. All participants were informed about the purpose and design of the study, and a written informed consent was signed by each subject. The study was in agreement with the declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University, School of Medicine.

2.2. Ultrasound

Abdominal ultrasound was performed in all subjects by experienced ultrasonographists. Normal liver was defined when the kidney cortex and liver presented a same parenchyma echogenicity. Otherwise, hyperechogenic liver with fine, tightly packed echoes was defined as steatosis.

In addition, the severity of NAFLD was classified into three grades according to the degree of steatosis under ultrasound: mild: a minimal discrepancy between hepatic and renal echoes, and normal intrahepatic vessel structures; medium: a greater exaggeration of discrepancy between liver and kidney echoes, greater posterior beam attenuation, mild deterioration in the contours of diaphragm and intrahepatic vessels; severe: apparent increase in hepatic echo, vague or disappeared diaphragm contours and intrahepatic vessel structure [14,15].

2.3. Serum Nrg4 Measurement

Fasting blood samples were collected from each subject and then stored at −80 °C. When performing the assay, samples were brought to room temperature. Serum Nrg4 levels were measured by using enzyme-linked immunosorbent assay (ELISA) (Catalogue No. SEC174Hu; USCN Life Science, China). The ELISA kit was a sandwich enzyme immunoassay for in vitro quantitative measurement of Nrg4 in human serum, plasma, tissue homogenates and other biological fluids. The intra-assay coefficient of variation (CV) of the kit was less than 10%, and the inter-assay CV was less than 12%.

2.4. Anthropometric and Biochemical Data

Data regarding to age, gender, height, weight and waist circumference (WC) were collected and recorded. BMI was calculated as the weight (in kilograms) divided by the square of the height (in meters). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by using a sphygmomanometer with the participant in a sitting position. At the same time, blood biochemical analyses were performed using a Hitachi 7600 AutoAnalyzer (Hitachi, Tokyo, Japan), while AFP levels were evaluated by an Abbott-Architect Immunoanalyzer (Abbott Laboratories, Abbott Park, IL). Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), gamma-