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Plasma osteoprotegerin levels are inversely associated with nonalcoholic fatty liver disease in patients with type 2 diabetes: A case–control study in China

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ABSTRACT

Objective. Osteoprotegerin (OPG), a soluble member of the tumor necrosis factor (TNF) receptor superfamily, is a decoy receptor for the receptor activator of nucleus factor-κB ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL). OPG has an effect on systemic insulin sensitivity and glucose homeostasis. The objective of this study was to evaluate the relationship between plasma osteoprotegerin (OPG) levels and nonalcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes.

Materials/methods. A case–control study was performed with 746 patients with type 2 diabetes. Of the study population, 367 patients had B-mode ultrasound-proven NAFLD and 379 were controls. The plasma OPG levels were measured using ELISA methods. NAFLD was diagnosed by hepatic ultrasound after the exclusion of alcohol abuse and other liver diseases.

Results. The OPG levels were significantly decreased in patients with NAFLD (2.3 ± 1.1 μg/L vs. 2.8 ± 1.3 μg/L, $p = 3.75 \times 10^5$) compared to controls. Pearson correlation analysis showed that the OPG levels were associated with age and systolic blood pressure (both $p < 0.05$). The participants in the lowest OPG quartile had a significantly increased risk for NAFLD (OR = 3.49, 95% CI 1.86–6.94) after adjusting for potential cofounders.

Conclusions. The plasma OPG level is negatively associated with NAFLD independent of potential cofounders.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive hepatic fat accumulation in patients who have no history of alcohol abuse [1]. NAFLD is strongly linked to insulin resistance, type 2 diabetes and obesity and is prevalent in up to 95% of obese patients and up to 70% of people with type 2 diabetes [2]. Because metabolic syndrome

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(MetS) is present in 30–88% of NAFLD patients, depending on which internationally recognized MetS diagnostic criteria are used, as well as severity of the disease [3,4], NAFLD is commonly regarded as the hepatic expression of MetS.

Osteoprotegerin (OPG), a soluble glycoprotein that belongs to the tumor necrosis factor (TNF) receptor superfamily, is produced by a variety of tissues, including the heart, arteries, veins, lung, kidney, immune system and bone [5]. OPG was originally discovered as an inhibitor of bone resorption, and various cytokines and hormones regulate its expression and production [1,5]. In addition to its crucial role in the regulation of bone metabolism, osteoprotegerin has important anti-inflammatory and anti-apoptotic effects [6]. It inhibits the activation of specific proinflammatory and proapoptotic signaling pathways by neutralizing the effect of receptor activator of nuclear factor-κ B ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL) [7,8]. The biological links between OPG and inflammation suggest that particular caution should be applied to the causative role of the OPG-RANKL system in metabolic disease [9].

Because the anti-inflammatory and anti-apoptotic effects of OPG are regulated by the RANKL and TRAIL signaling pathways, there is interest in determining the association between OPG and NAFLD. Several epidemiologic studies [10,11] suggested that circulating OPG levels are decreased in NAFLD subjects, although data from another study are inconsistent [12]. Furthermore, recent studies also demonstrated the association of OPG with insulin resistance and type 2 diabetes [13–15]. Based on these observations, we hypothesize that there is a pathogenic interplay of OPG, liver lipid accumulation and abnormal glucose metabolism.

Therefore, the objective of this study is to assess the association between plasma OPG levels and NAFLD in a large cohort of type 2 diabetic adults.

2. Methods

2.1. Study Population

Subjects were recruited from the Department of Endocrinology at Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine between 2013 and 2014. All unrelated subjects with T2DM who attended the Diabetes Clinic at the Xinhua Hospital were recruited consecutively to participate in a prospective study to identify the risk factors predisposing patients to the development of diabetic complications. Each visit was composed of clinical assessments and laboratory investigations to determine the control of diabetes and related cardiovascular risk factors as well as the presence of diabetic complications. Diabetes was defined according to the 2008 American Diabetes Association diagnostic criteria (MM) [16]. All subjects received a hepatic ultrasonographic examination. Subjects without any of the following conditions were selected for this study: severe psychological disorders, physical disabilities, cancer, senile dementia or currently diagnosed with tuberculosis, acquired immune deficiency syndrome, clinical signs or symptoms of inborn errors of metabolism, a history of use of toxins or drugs associated with liver steatosis or any other communicable disease. Elevated liver enzymes (serum alanine aminotransferase [ALT], aspartate aminotransferase [AST] and gamma glutamyltransferase [GGT]) were not exclusion criteria. Fatty liver patients consuming more than 20 g of alcohol per day were excluded. A total of 746 T2DM subjects who attended regular visits at least twice a year with the latest follow-up on or before April 2014 were enrolled in the study. Written informed consent was obtained from all participants. The Institutional Review Broad of Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine approved this study.

2.2. Clinical Data Collection and Biochemical Measurements

Anthropometric parameters were measured in all subjects. The details of the anthropometric measurements, including the waist circumference (WC), were obtained by trained physicians. The waist circumference was defined as the midway level between the costal margins and the iliac crests. Blood pressure was assessed twice at the right arm after a 15-min rest in a sitting position using a standard mercury sphygmomanometer. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Age, smoking (yes/no, current or former smoking defined as “yes”; never smoking defined as “no”) and alcohol drinking (yes/no, drinking but alcohol consumption less than 20 g per day defined as “yes”; no drinking defined as “no”) were assessed using an interview preceding the physical examination. The physical activity level was classified as low, moderate, or high according to the International Physical Activity Questionnaire scoring protocol.

Peripheral venous blood samples were drawn after overnight fasting for 10 h and were centrifuged at 3000 rpm for 10 min within the first hour of collection, and the isolated plasma samples were stored at −80 °C until assayed. All subjects underwent a standardized clinical and laboratory evaluation. The homeostasis model assessment (HOMA) value for insulin resistance (HOMA-IR) was estimated as previously described with the following formula: fasting insulin × fasting glucose/22.5. The HOMA-β was calculated using the formula described by Matthews et al. [17].

2.3. Plasma OPG Levels

The plasma OPG was determined in duplicate by ELISA with the Duoset kit (DY805; R&D Systems, Minneapolis, MN) as recommended by the manufacturer. The ELISA system had an intraassay coefficient of variation of 3–9% and an interassay coefficient of variation of 4–10.2%.

2.4. Fatty Liver Measurement

Hepatic ultrasonographic examination was performed by experienced ultrasonographists using a high-resolution B-mode tomographic ultrasound system (Esaote Biomedica SpA) with a 3.5-MHz probe.

2.5. Statistical Analysis

The clinical and laboratory values are shown as the means ± SD or as the median with the interquartile range. Comparisons of the clinical and laboratory parameters between the NAFLD and