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Original Article

Correlation of increased plasma osteoprotegerin and cardiovascular risk factors in patients with adult growth hormone deficiency

Jiajia Xia, Linman Li, Wei Ren, Xiaoya Zheng, Chan Liu, Jinchao Li, Tingying Chen, Xiaofeng Li, Lingli Wang, Yumei Hu

Department of Endocrinology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, P. R. China

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Abstract: Adult growth hormone deficiency (AGHD) is correlated to many adverse effects on metabolism and increases cardiovascular risk. 40 patients with AGHD and 40 healthy subjects were included. Anthropometric parameters such as body mass index, waist circumference, and waist-hip ratio were measured. Meanwhile, plasma levels of total cholesterol, triglyceride, high sensitivity C-relative protein, interleukin-6 and OPG were determined. Homeostasis model assessments for insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were calculated using homeostasis model. Plasma OPG concentrations of AGHD patients were significantly higher than those in healthy subjects (131.82 ± 45.04 versus 81.02 ± 45.04, $P < 0.01$). Plasma OPG levels were positively correlated with age, body mass index, waist circumference, hip circumference, waist-hip ratio, fasting insulin, total cholesterol, triglyceride, high sensitivity C-relative protein and interleukin-6 ($P < 0.05$), but negatively correlated with high-density lipoprotein cholesterol ($P < 0.05$). Multiple linear stepwise regression analysis demonstrated that body mass index, triglyceride, and interleukin-6 were independently related to plasma OPG levels ($P < 0.05$). The levels of plasma OPG were increased in AGHD patients and were closely correlated with glycolipid metabolism and chronic inflammation. OPG might play an important role in the occurrence and development of cardiovascular diseases in AGHD patients.

Keywords: Adult growth hormone deficiency, osteoprotegerin, insulin tolerance test, cardiovascular disease risk

Introduction

Adult growth hormone deficiency (AGHD) is correlated to many adverse effects on metabolism and increases cardiovascular risk. AGHD causes some complications, such as abnormal body composition [1, 2], decreased bone mineral content and density [3], bone fracture [4], abnormal glucolipid metabolism and chronic low-grade inflammation [5-8]. Several prospective studies have confirmed that AGHD significantly increases mortality rate of patients, especially those with cardiovascular diseases [9]. Side effects and bad results can be reversed by adopting reasonable replacement therapy in time. Epidemiological studies have shown that osteoporosis is closely correlated to atherosclerosis, and elevated osteoprotegerin (OPG) levels increase the risk of carotid intima-media thickening and osteoporosis at the same time [10, 11]. Clinically, patients with chronic inflammation, postmenopausal women and old people are often affected by atherosclerosis and osteoporosis. Some researchers suggest that there may be a common pathway that plays important roles in bone metabolism and cardiovascular diseases.

OPG, also known as osteoclastogenesis inhibitory factor (OCIF) and tumor necrosis factor (TNF) receptor superfamily member 11 b (TNFRSF11B), was first described as an important regulatory factor of bone metabolism [12]. OPG, a soluble glycoprotein composed of 380 amino acid residues, can be found in osteoblasts of the bone, vascular endothelial cells and smooth muscle cells [13]. OPG competes with receptor activator of nuclear factor kappa-B ligands (RANKL) that inhibit osteoclast differentiation and destruct bone absorption [14]. Previous studies have demonstrated that decreased gene expression of OPG causes severe

osteoporosis and unexpected vascular calcification. However, increased OPG gene expression can retard the loss of bone mass and the occurrence of atherosclerosis [15-17]. Some researchers explored the correlation between OPG levels and the risk factors of atherosclerosis and cardiovascular diseases [18-20]. Especially, higher plasma OPG levels were associated with increased risks of cardiovascular disease mortality [21]. Therefore, we hypothesize that the levels of OPG may be linked to bone and blood vessel diseases. AGHD population has a high risk of osteoporosis and cardiovascular disease. However, data on OPG in AGHD patients are limited, and the relationship between OPG and atherosclerosis in AGHD population is not well known. In this study, we investigate how plasma OPG levels are related to clinical parameters, glucolipid metabolism, insulin resistance and inflammation in patients with AGHD.

Materials and methods

Subjects

Between June 2009 and September 2012, 40 patients were diagnosed as AGHD, including 35 pituitary adenoma patients who were treated by operation over 6 months ago and 5 patients with Sheehan syndrome. All AGHD subjects were treated by adequate and stable hormone replacement therapy with gonadal, thyroid and glucocorticoid hormones for more than six months in addition to growth hormone when they needed the lack of hormones. All patients have undergone insulin tolerance test, which was defined as the gold standard for the diagnosis of AGHD [22]. When growth hormone secretion peak was < 5.0 μg/L, AGHD was diagnosed. According to this standard, we selected 40 patients into the AGHD group and 40 healthy subjects with matched age and gender into the control group. The exclusion criteria were: i) diabetes mellitus and acute and chronic complications of diabetes; ii) severe or sustained hypertension or heart diseases; iii) hepatic and renal diseases; iv) malignant tumors; v) epilepsy or a history of mental disorders; vi) treatment with lipid lowering medications; vii) active infections. The study was approved by the Ethics Committee of Chongqing Medical University. Written informed consents were obtained from all subjects in this study.

According to standardized instructions, body weight, height, waist and hip circumferences, body fat percentage and blood pressure were measured in all subjects. Body mass index (BMI) was calculated as weight per height squared (kg/m^2), and waist-hip ratio was evaluated as the ratio of waist and hip circumferences.

Samples

Overnight venous blood samples were collected from cubital vein for biochemical analysis after fasting for 10-12 h. A portion of the fresh serum was used for the determination of fasting plasma glucose (FPG), fasting insulin (FINS), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), while the remaining portion of the fresh serum was frozen at -80°C for later measurements of OPG, high sensitivity C-relative protein (hs-CRP) and interleukin-6 (IL-6) levels. FPG was measured using glucose oxidase method. FINS was assayed by radioimmunoassay using human insulin as standard (Linco, St. Charles, MO, USA). Lipid metabolism spectrums were detected by biochemical autoanalyzer (CX-7 Biochemical Autoanalyser; Beckman, Brea, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of OPG and IL-6 were measured using enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, Shanghai, China and CUSABIO Science Co., Ltd., China, respectively). Hs-CRP was detected by an enzymatic kit (Roche Diagnostics, Germany).

Homeostasis model assessment

Homeostasis model assessments for insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were calculated using the following equations: HOMA-IR = fasting insulin (mU/L) × [Fasting plasma glucose (mmol/L)/22.5], and HOMA-β = [20 × fasting insulin (mU/L)]/[fasting plasma glucose (mmol/L)-3.5].

Statistical analysis

All statistical analyses were performed using SPSS statistical software version 17.0 (SPSS, Inc., Chicago, USA). Normal distribution data

Table 1. Anthropometric parameters

Variables	Control group	AGHD group	*P* value
No. of patients (M/F)	40 (8/32)	40 (14/26)	
Age (years)	47.05 ± 11.65	51.75 ± 10.02	0.057
BMI (kg/m^2)	22.90 ± 3.34	25.33 ± 2.91	0.001*
Body fat percentage (%)	30.55 ± 4.96	30.95 ± 6.36	0.759
Waist circumference (cm)	75.00 ± 8.61	84.15 ± 10.18	0.000*
Hip circumference (cm)	91.73 ± 6.25	93.78 ± 9.39	0.254
Waist-hip ratio	0.82 ± 0.06	0.90 ± 0.10	0.000*
SBP (mmHg)	117.98 ± 15.88	122.00 ± 19.04	0.325
DBP (mmHg)	78.00 ± 9.15	77.83 ± 12.00	0.924

Note: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. Data are presented as means ± SD. *$P < 0.01$ compared with control group.

Table 2. Metabolic parameters

Variables	Control group	AGHD group	*P* value
FPG (mmol/L)	5.39 ± 0.350	5.06 ± 0.76	0.017*
FINS (mU/L)	4.58 ± 1.80	8.26 ± 1.80	0.000#
HOMA-β	48.17 (34.64, 62.17)	100.86 (61.11, 155.88)	0.899
HOMA-IR	1.10 ± 0.45	1.93 ± 0.50	0.000#
TC (mmol/L)	4.78 ± 0.84	6.32 ± 0.86	0.000#
TG (mmol/L)	1.36 ± 0.55	1.70 ± 0.73	0.023*
HDL-C (mmol/L)	1.40 ± 0.31	1.04 ± 0.42	0.000#
LDL-C (mmol/L)	2.57 ± 0.80	2.72 ± 0.93	0.422
hs-CPR (mg/L)	0.48 (0.31, 0.89)	1.73 (0.56, 2.88)	0.000#
IL-6 (pg/ml)	1.50 (1.10, 1.70)	5.74 (4.57, 6.92)	0.000#
OPG (ng/L)	96.51 ± 28.40	124.70 ± 47.06	0.000#

Note: FPG, fasting plasma glucose; FINS: fasting insulin; HOMA-β, homeostasis model assessment index for β-cell function (insulin secretion); HOMA-IR, homeostasis model assessment index for insulin resistance; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; hs-CPR, high sensitivity C-reactive protein; IL-6, interleukin-6; OPG, osteoprotegerin. Normal distribution of data was expressed as means ± SD. Skewed distribution of data was expressed as median and interquartile ranges, and log-transformed (base 10) before statistical analysis. *, $P < 0.05$; #, $P < 0.01$ compared with control group.

were presented as means ± standard deviation (SD). Skewed distribution data were expressed as medians with interquartile range and were log-transformed (based on 10) before analysis. Independent sample *t*-tests were used to compare continuous variables between 2 groups. The interrelationships between OPG and other variables were analyzed by Pearson correlation analysis. Independent predictors of plasma OPG were analyzed by multiple linear regression analysis. For all statistical analyses, *P* values < 0.05 were considered statistically significant.

Results

AGHD alters the anthropometric and metabolic parameters of patients, as well as plasma OPG concentration

To determine how AGHD affects patients, we measured anthropometric and metabolic parameters. The data showed that no significant difference was found between AGHD and control groups in age, weight, body fat percentage, hip circumference, systolic pressure and diastolic pressure ($P > 0.05$). Compared to the control group, AGHD group exhibited higher levels of BMI, waist circumference, and waist-hip ratio ($P < 0.05$) (**Table 1**). In addition, there was no significant difference in HOMA-β and LDL-c between AGHD and control groups ($P > 0.05$). Compared to the control group, the AGHD group exhibited higher levels of FINS, HOMA-IR, TC, TG, HDL-c, hs-CRP and IL-6 ($P < 0.05$), but lower levels of FPG ($P < 0.05$) (**Table 2**). Moreover, plasma OPG concentration in AGHD group was significantly higher than that in the control group ($P < 0.01$) (**Figure 1**). These data suggested that AGHD altered the anthropometric and metabolic parameters of patients, as well as plasma OPG concentration.

Plasma OPG levels are significantly correlated with anthropometric and metabolic parameters

To investigate whether plasma OPG levels are correlated with anthropometric or metabolic parameters, bivariate correlation analysis was performed. The results revealed that plasma OPG levels were positively correlated with age, BMI, waist circumference, hip circumference, waist-hip ratio, FINS, HOMA-IR, TC, TG, hs-CRP and IL-6 (*r* values were 0.377, 0.312, 0.431, 0.244, 0.361, 0.398, 0.423, 0.371, 0.335,

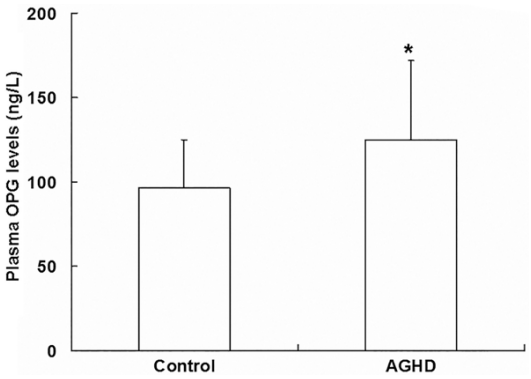

Figure 1. Plasma OPG levels in control and AGHD groups. The concentration of OPG was measured using ELISA according to the manufacturer's instructions. Data are means ± SD. *, $P < 0.01$ compared with control group.

Table 3. Correlation of plasma OPG levels to anthropometric and metabolic parameters

Indicators	Pearson value (r_s)	*P* value
Age (years)	0.377	0.001#
BMI (kg/m^2)	0.312	0.005#
Waist circumference (cm)	0.431	0.000#
Hip circumference (cm)	0.244	0.029*
Waist-hip ratio	0.361	0.001#
FINS (mU/L)	0.398	0.000#
HOMA-IR (mmol/L)	0.423	0.000#

Note: *, $P < 0.05$; #, $P < 0.01$ compared with control group.

0.361 and 0.469, respectively; $P < 0.05$), but negatively correlated with HDL-c (*r* value was -0.246; $P < 0.05$). However, plasma OPG levels were not significantly correlated with body fat percentage, blood pressure, FPG, HOMA-β or LDL-c ($P > 0.05$) (**Table 3**; **Figure 2**). Furthermore, multivariable linear regression models revealed that BMI, TG and IL-6 were independently related with plasma OPG levels (β = 0.232, 0.304, and 0.300, respectively; $P < 0.05$). The multiple regression equation was: $Y_{OPG} = -3.039 + 0.232X_{BMI} + 0.304X_{TG} + 0.300X_{IL-6}$. These data indicated that plasma OPG levels were significantly correlated with anthropometric and metabolic parameters.

Discussion

OPG exerts its effects mainly by binding to RANKL and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and hence, inhibiting a series of biological effects. For example, OPG inhibits the regulatory effects of nuclear factor kappa-B (NF-κB) on inflammation in skeletal and vascular systems, and prevents TRAIL-induced apoptosis. Bone tissue OPG is mainly produced by osteoblasts and vascular OPG is mainly produced by endothelial and smooth muscle cells. Lacey et al. [23] discovered that the cognate ligand of OPG is RANKL that binds to the membrane of bone forming cells, immune cells and cancer cells. NF-κB can increase the expression of proto-oncogene c-Foc, which can promote osteoclast gene transcription after the activation of T cell nucleus factor c1 (NFAT-c1) [24]. OPG is an important regulatory factor of bone metabolism, but some studies show that OPG is also a kind of important vascular regulatory factor that is closely associated with atherosclerosis and vascular calcification, suggesting that OPG plays a vital role in the pathophysiological process of cardiovascular diseases [25-28].

Growth hormone has extensive and complex biological effects, such as promoting growth and development in children and regulating metabolism in adults. Lack of growth hormone can influence body composition, lipid metabolism, glucose metabolism, bone metabolism and the quality of life. Patients with growth hormone deficiency often show decreased muscle and increased fat content, leading to disordered ratio of adipose to lean tissue [22].

Our study showed that BMI, waist circumference, hip circumference and waist-hip ratio of AGHD patients were higher than those of healthy subjects with matched age and gender, but no significant difference was observed in body fat percentage. These results indicated that fat tissue, especially the abdominal fat tissue, was increased. Some studies suggested that obesity and increased waist-hip ratio could be used as the predictors for atherosclerosis [29] and cardiovascular events [30]. Chagas et al. [31] studied 337 patients with coronary heart disease and used Friesinger Score to evaluate the severity of coronary lesions. Their study showed that higher FS score corresponded to heavier degree of coronary artery lesions. Therefore, waist-hip ratio was significantly associated with FS score, indicating that adipose tissue position was more important than obesity itself. Our study showed that the waist-hip ratio in AGHD group was significantly higher than that in control group, suggesting that increased incidence of cardiovascular events

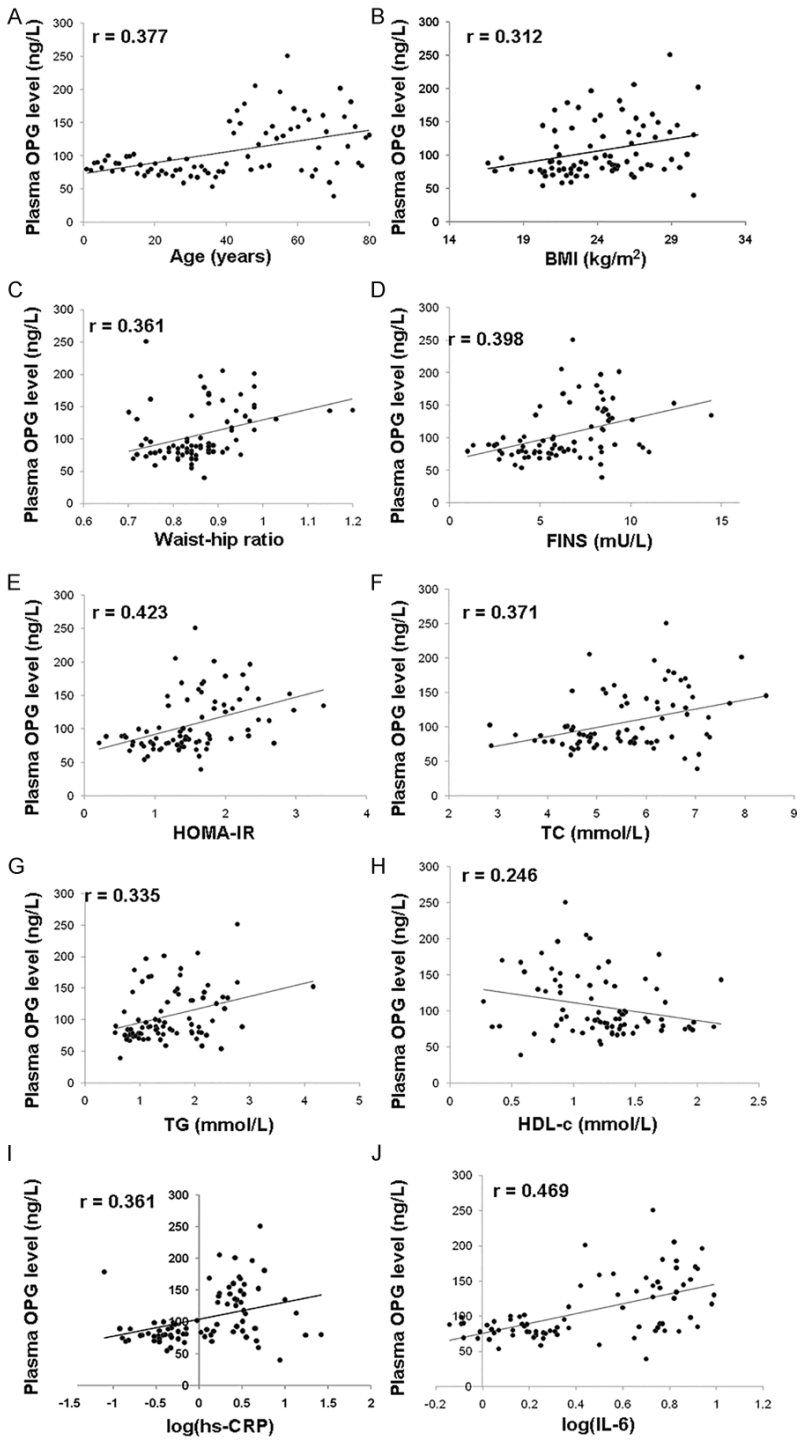

Figure 2. Correlation between plasma OPG levels and (A) Age, (B) BMI, (C) waist-hip ratio, (D) FINS, (E) HOMA-IR, (F) TC, (G) TG, (H) HDL-c, (I) hs-CRP and (J) Log(IL-6). The interrelationships between OPG and other variables were analyzed by Pearson correlation analysis. $P < 0.05$ for all parameters. IL-6 was transformed by natural logarithm.

might be related to increased waist-hip ratio. Our study also showed that plasma OPG level was significantly correlated to BMI, waist circumference, hip circumference and waist-hip ratio in the AGHD group. These results supported the hypothesis that plasma OPG level could be considered as a vascular risk factor in AGHD patients.

AGHD patients were usually thought to exhibit increased insulin sensitivity. However, Hew and his colleagues [32] found reduced glucose utilization and glycogen synthesis in AGHD patients, leading to increased blood glucose and insulin resistance. Arumugam et al. [33] showed that the mouse model with growth hormone deficiency exhibited insulin resistance through the dysregulation of insulin secretion. Our results showed that fasting blood glucose was lower, and fasting insulin (FINS) and HOMA-IR were higher in AGHD group than in control group, while HOMA-β was not significantly different between the two groups. Correlation analysis showed that plasma OPG levels were positively correlated with FINS and HOMA-IR, but slightly negatively correlated with HOMA-β ($r = 0.200$, $P = 0.075$). These results indicated that circulating OPG levels were significantly positively correlated with insulin resistance and negatively correlated with pancreatic β-cell function. However, the specific reason why the two groups had different postprandial insulin levels was yet to be determined. In terms of glucose metabolism, growth hormone has the function

to increase blood glucose utilization. Therefore, children who lack of growth hormone often have low blood sugar. Our study showed that AGHD group had lower FPG compared with control group. This may be related to the role of growth hormone antagonist insulin. These results suggested that OPG might be closely correlated to glucose metabolism and insulin resistance in AGHD patients.

A retrospective study of 110 AGHD patients showed that 41% of the patients had hypercholesterolemia, 41% of the patients had high triglyceride levels, 48% of the patients had elevated LDL-c concentrations and 47% had lower HDL-c levels [34], suggesting that AGHD patients were prone to have blood lipid spectrum disorder. Consistently, study by Barter et al. [35] showed significantly increased triglycerides and LDL-c, whereas HDL-c was significantly decreased in patients without reasonable growth hormone replacement therapy. An epidemiological survey indicated [35] that coronary heart disease or cardiovascular events would still occur even if 2/3 of patients with coronary heart diseases had well controlled blood LDL-c levels. Therefore, low level of HDL-c is another important cause of cardiovascular events in addition to other risk factors. A 5-year follow-up study conducted by Despres et al. [36] showed that decrease of HDL-c levels by 10% led to 13% increase of the risk of coronary heart disease. Therefore, reducing HDL-c levels may have an important effect on the occurrence of cardiovascular events. The above research results were consistent with our study that showed significantly increased total cholesterol and triglyceride levels, and decreased HDL-c levels in AGHD group. Triglycerides, total cholesterol and HDL-c were correlated with OPG. It is well known that abnormal lipid metabolism is one of the most important risk factors for atherosclerosis. A significant increase in the levels of plasma OPG and an alteration in lipid profile suggest a possible link between OPG levels and atherosclerosis in AGHD patients.

AGHD patients are associated with the state of chronic low-grade inflammation, which is characterized by increased pro-inflammatory factors and decreased anti-inflammatory factors. A growing number of studies showed inflammatory factors play important roles in the process of occurrence and development of cardiovascular and cerebrovascular diseases [7]. One of the major inflammatory markers, hs-CRP is considered to be an independent risk factor for atherosclerosis and cardiovascular diseases. A prospective study for 20 years [37] showed that elevated serum hs-CRP levels in healthy people could predict significantly increased risk of cardiovascular diseases. Recently, a report [38] showed that increased IL-6 expression by the inhibition of tyrosine phosphorylation of insulin receptor substrate, led to decreased insulin secretion as well as insulin resistance. Our study showed significantly increased serum hs-CRP and IL-6 levels in AGHD patients. In addition, we found that plasma OPG levels were significantly positively correlated with hs-CRP and IL-6 levels. Correct treatment could reduce the levels of inflammatory biomarkers and hence, increasing the function of β-cells [39]. Higher hs-CRP and IL-6 levels in AGHD patients suggested that low-grade chronic inflammation might be involved in the development of atherosclerosis and coronary heart diseases in AGHD population.

Researches on the relationship between coronary heart disease and peripheral artery atherosclerosis showed that plasma OPG levels were not only significantly associated with the presence of atherosclerosis, but also significantly correlated with its severity [40]. High plasma OPG level is an independent risk factor for heart disease and cardiovascular disease. Therefore, OPG levels can be used for risk assessment of coronary artery pathological changes.

Bone metabolic abnormalities are commonly observed on AGHD patients, with high cardiovascular morbidity and mortality. Many studies showed that reduced bone mineral density, osteoporosis and bone fracture could easily occur in AGHD population [41]. The mechanism may be related to reduced bone turnover and bone mineral content [42]. Our research showed that plasma OPG levels were significantly higher in AGHD patients than in healthy subjects, being associated with metabolic disorder and cardiovascular risk factors.

Despite limited number of samples, our study still revealed: i) OPG, BMI, waist circumference, hip circumference, waist-hip ratio, FINS, HOMA-IR, TC, TG, hs-CRP and IL-6 were increased while HDL-c was decreased in AGHD patients; ii) Plasma OPG level was positively correlated

to age, BMI, waist circumference, hip circumference, waist-hip ratio, FINS, HOMA-IR, TC, TG, hs-CRP and IL-6, but negatively correlated to HDL-c in AGHD patients; iii) BMI, TG and IL-6 were independent correlative factors for OPG in AGHD patients.

By now, there are some limitations in our study. First, the number of samples was small, and insignificant associations between OPG and some factors might become statistically significant if large number of samples were enrolled. Second, our study is a cross-sectional research, in which the causality of OPG and the inflammatory biomarkers could not be explained. Third, further study is needed to determine whether reducing plasma OPG levels can diminish the severity of atherosclerosis and reduce the occurrence of cardiovascular diseases.

In conclusion, we demonstrated the increase of plasma OPG levels in AGHD patients. The levels of plasma OPG were correlated closely with glycolipid metabolic disorder, chronic inflammation and insulin resistance in AGHD population, suggesting that OPG might contribute to the pathogenesis of cardiovascular diseases in AGHD patients. Therefore, OPG could be a potential therapeutic target for the treatment of cardiovascular diseases.

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Address correspondence to: Wei Ren, Department of Endocrinology, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing 400016, P. R. China. Tel: +86-23-89012496; Fax: +86-23-89013960; E-mail: weiren1967@yeah.net

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