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ORIGINAL ARTICLE

Increase in serum pregnancy-associated plasma protein-A is correlated with increase in cardiovascular risk factors in adult patients with growth hormone deficiency

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Abstract Adult Growth Hormone Deficiency (AGHD) is correlated to many adverse effects on metabolism and increased cardiovascular risk. Pregnancy-associated plasma protein-A (PAPP-A) is a protease that promotes IGF-I availability in vascular tissues in recent study, and PAPP-A levels have been proposed as an early predictor of cardiac events. The aim of our study was to compare PAPP-A levels in AGHD patients with that of healthy adult subjects to determine if there is a relationship between serum PAPP-A and glucose and lipid metabolism. Twenty AGHD patients and 20 healthy, age-matched and weight-matched persons were chosen for the study. Their weight, height, blood pressure, body mass index (BMI), body fat percentage, waist and hip circumference, and waist–hips ratio were assessed. An oral glucose tolerance test was performed and venous blood was collected from the each patient's cubital vein for biochemical analysis. Serum PAPP-A level in AGHD patients was significantly higher than that of the control group [(7.62 ± 1.62 *vs.* 6.54 ± 1.31) $p < 0.05$], and PAPP-A was positively correlated to age, BMI, waist circumference and so on. After adjusting for the waist circumference, waist–hip ratio, 2 h postprandial blood glucose, triglycerides, the serum PAPP-A in AGHD patients was positively correlated to the BMI ($r = 0.728$, $p < 0.05$) and fasting insulin ($r = 0.433$, $p < 0.05$). In a multiple step-wise regression analysis, BMI, 2 h postprandial glucose, fasting insulin, HOMA-IR were independently associated with serum PAPP-A in AGHD patients. The increase in serum PAPP-A levels is associated with abnormal glucose metabolism and increased risk of atherosclerosis in AGHD patients.

Keyword Adult growth hormone deficiency · Serum pregnancy-associated plasma protein-A · Insulin tolerance test

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Introduction

Several studies have shown that adult growth hormone deficiency (AGHD) influences body composition, quality of life, and significantly increases the mortality rate in patients suffering from cardiovascular diseases [1–6]. In addition to hypertension, obesity, diabetes mellitus, and lack of exercise, AGHD could also increase the risk of cardiovascular disease. AGHD is associated with increase in body mass index (BMI), waist–hip ratio, arterial thrombophilia caused by an increase in plasminogen activity inhibitor (PAI-1), and fibrinogen [7], an abnormal lipid profile and atherosclerosis [8, 9]. There is evidence of increased risk of common carotid atheromatous plaque and carotid intima-media thickening in AGHD patients without growth hormone replacement therapy [8, 10]. Pregnancy-associated plasma protein-A (PAPP-A) is closely related to atherosclerosis and carotid intima-media thickness [11, 12]. Therefore, it is recommended as an independent marker for early diagnosis and prognosis of acute coronary syndrome (ACS) [13]. PAPP-A exerts its biological effects by acting on the growth hormone/insulin-like growth factors-1 axis (GH/IGF axis). The severe GH deficiency in AGHD patients leads to a decrease in insulin-like growth

factors-I (IGF-1). Whether or not PAPP-A level is changed, and whether or not the expected change is related to IGF-1 in AGHD patients remain to be clarified. Current research literatures on PAPP-A in the AGHD population is sparse. The aim of our study is two-fold: (1).To evaluate the changes in PAPP-A levels in relation to the changes in IGF-1 and several cardiovascular diseases risk factors including BMI, blood glucose, blood lipids, blood pressure, insulin, high-sensitivity C-reactive protein (CRP), and free fatty acid (FFA) in AGHD patients. (2).To confirm whether the PAPP-A in AGHD patients, whose main clinical manifestations is atherosclerosis, is linked to increased risk of cardiovascular disease.

Subjects and methods

Subjects

The research subjects included 19 patients who were over 6 months post-operation for pituitary adenoma and two patients who were diagnosed with Sheehan syndrome for further consulting from June 2009 to August 2010. All subjects were between 27 and 69 in age and were our hospital's patients between June 2009 and August 2010. All subjects received adequate and stable replacement therapy with gonadal, thyroid and/or glucocorticoid hormones, when needed. According to the insulin tolerance test, the gold standard for diagnoses of adult GH deficiency [14], those with GH peak value <5.0 μg/L were diagnosed as AGHD. According to this standard, a total of 20 patients were included in this study and one was excluded. The one excluded from the study was a patient with pituitary non-functioning adenoma. Those 20 included in the study were diagnosed with AGHD whose panhypopituitarism was due to pituitary non-functioning adenoma ($n = 13$), prolactinoma ($n = 1$), mixed pituitary adenoma ($n = 2$), craniopharyngioma ($n = 2$), and Sheehan syndrome ($n = 2$). These 20 subjects were assigned to the AGHD group and 20 age-matched healthy subjects were used as controls. The characteristics of these 40 subjects are described in Table 1. Written consent was obtained from all subjects. The criteria for exclusion from the study for both groups were a history of malignant tumor, diabetes mellitus, heart diseases, liver and kidney functional disorders, severe hypertension or mental disorder.

Table 1 Comparison of clinical and biochemical indexes

	AGHD group	Control group	*p* value
Patients no. (M/F)	20 (7/13)	20 (7/13)	
Age (years)	51.4 ± 11.6	51.5 ± 13.6	0.980
BMI (Kg/m^2)	23.5 ± 3.3	22.9 ± 2.8	0.503
Body fat percentage	32.3 ± 4.8	27.6 ± 7.1	0.023
Waist circumference (cm)	82.25 ± 10.45	76.65 ± 9.17	0.019
Waist–hip ratio	0.91 ± 0.10	0.85 ± 0.07	0.029
SBP (mmHg)	117 ± 14	114 ± 14	0.512
DBP (mmHg)	76 ± 11	73 ± 8	0.239
FPG (mmol/L)	5.34 ± 0.60	5.31 ± 0.51	0.843
2hPG (mmol/L)	7.50 ± 1.81	6.25 ± 1.13	0.014
FINS (mU/L)	4.89 (1.17–14.42)	6.33 (2.87–15.36)	0.092
HOMA-β	47.59 (11.13–161.12)	6 67.94 (38.27–183.7)	0.588
HOMA-IR	1.16 (0.24–3.39)	1.50 (0.56–3.62)	0.10
TC (mmol/L)	5.31 ± 1.36	4.59 ± 0.56	0.039
TG (mmol/L)	1.80 ± 0.87	1.19 ± 0.38	0.007
HDL-C (mmol/L)	1.17 ± 0.29	1.41 ± 0.33	0.021
LDL-C (mmol/L)	2.96 ± 0.96	2.51 ± 0.48	0.085

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting serum glucose, *2hPG* 2 h postprandial glucose, *FINS* fasting serum insulin, *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

Methods

Prior to the specimen collection, basic physical parameters including weight, height, blood pressure, BMI, body fat percentage, waist and hip circumference, and waist–hips ratio were evaluated. Oral glucose tolerance test was performed after a 10–12 h fast and venous blood was collected from the cubital vein for biochemical analysis. A portion of the fresh serum was used for analysis of glucose, insulin, and lipids while the remaining portion of the fresh serum was stored at −80 °C and later evaluated for PAPP-A, IGF-1, FFA, and CRP levels. PAPP-A was measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Shanghai, PRC). Serum lipids and CRP were measured by an enzymatic kit (Roche Diagnostics, Germany). Serum glucose was measured by a glucose oxidase kit (BIOSINO BIO-TEC). Serum insulin and IGF-1 were assessed by an assay using chemiluminescence (Roche Diagnostics, Germany). This assay adopts homeostatic model to assess insulin resistance index (HOMA-IR = FPG × FINS/22.5) and insulin secretion index [HOMA-β = FINS × 20/(FPG-3.5)].

Statistics

All data were expressed in mean ± standard deviation (SD) and $p < 0.05$ was deemed statistically significant. Comparison between the two groups of subjects was determined using paired *t* test. Abnormal data were

analyzed by the rank sum test to confirm continuous distribution in both groups. The inter-index relation was determined by Pearson correlation, partial correlation, and multiple linear regression analysis. All statistical analyses were performed using Version 13.0 of statistical package for the social sciences (SPSS).

Results

Comparison of the clinical and biochemical indexes between the AGHD group and the control group

As shown in Table 1, body fat percentage, waist circumference, waist–hip ratio, 2 h postprandial blood glucose, total cholesterol, and triglycerides in AGHD patients were higher than those in the control group ($p < 0.05$). While the levels of high-density lipoprotein in the AGHD patients were lower than those in the control group ($p < 0.05$), the other variables (age, BMI, systolic blood pressure, diastolic blood pressure, fasting blood glucose, fasting insulin, HOMA-IR, HOMA-β, and low-density lipoprotein) were not significantly different between the two groups ($p > 0.05$).

Comparison of serum PAPP-A levels, IGF-1, FFA, and CRP between the two groups

As shown in Table 2, both PAPP-A levels and CRP were significantly higher in the AGHD group compared to that of the control group ($p < 0.05$). IGF-1 was significantly lower compared to that of the control group ($p < 0.05$). There was no difference between the two groups in the FFA level.

Correlation analysis

As shown in Fig. 1, the serum PAPP-A in AGHD patients was positively correlated to age, BMI, waist circumference, waist–hip ratio, systolic blood pressure, 2 h postprandial blood glucose, fasting insulin, and HOMA-IR (r value was 0.451, 0.878, 0.813, 0.605, 0.489, 0.549, 0.503, and 0.487, respectively; $p < 0.05$). There was no significant correlation to fasting blood glucose, diastolic blood pressure, total cholesterol, low-density lipoprotein, high-density lipoprotein, CRP, FFA, and IGF-1. After adjusting for waist circumference, waist–hip ratio, 2 h postprandial blood glucose, triglycerides, the partial correlation analysis showed that PAPP-A remains positively correlated to BMI and fasting insulin (r value was, respectively, 0.728 and 0.433; $p < 0.05$).

Table 2 Comparison of PAPP-A, IGF-1, FFA, and CRP between the two groups

	AGHD group	Control group	p value
CRP (mg/L)	1.21 ± 0.83	0.54 ± 0.26	0.012
FFA (μmol/L)	520.5 ± 235.9	500.1 ± 198.5	>0.05
IGF-1 (ng/ml)	120.4 ± 80.9	183.8 ± 63.3	0.019
PAPP-A (mIU/L)	7.62 ± 1.62	6.54 ± 1.31	0.026

CRP C-reactive protein, *FFA* free fatty acid, *IGF-1* insulin-like growth factors-1, *PAPP-A* pregnancy-associated serum protein-A

Discussion

The primary source of PAPP-A is the serum of pregnant women. However, recent studies have shown that PAPP-A is also present in the serum of non-pregnant women as well as men. Notable levels of PAPP-A is present in human fibroblast [15], macrophage, osteoblast, marrow stromal cell [16], vascular smooth muscle cell, and unstable atheromatous plaque [17]. Recent studies suggest that PAPP-A is a member of the matrix metalloproteinases (MMPs) family, an ion-dependent, zinc finger of the metalloproteinase [18]. In the presence of IGF-1 and IGF-2, PAPP-A specifically slices IGFBP-4 into two smaller fragments. The fragments are unable to bind to IGF-1 and IGF-2, therefore increasing the release of free IGF-1 and IGF-2, and enhancing their biological effects [15].

The primary source of circulating IGF-1 is the interstitial cells of the liver. The circulating IGF-1 levels are regulated primarily by growth hormone. Growth hormone stimulates the secretion of IGF-1 from the liver via its action on the growth hormone receptor. Our findings show that the concentration of serum IGF-1 is decreased and serum PAPP-A is increased in AGHD patients. PAPP-A exerts its biological effect by acting on GH/IGF axis. However, whether the rise of serum PAPP-A is a reaction to the decreased IGF has not been substantiated. Neither our study was unable to establish a correlation between the two. Our finding is consistent with the report from Joaquin C [19], in which 14 cases of AGHD were studied. IGF-1 levels increased with growth hormone replacement therapy while PAPP-A decreased. However, the level of IGF-1 and PAPP-A were uncorrelated before or after the growth hormone therapy. Perhaps the rise of serum PAPP-A was not a reaction to the decreased IGF as was envisaged but may be a reaction to an elevated level of oxidative stress and endothelial dysfunction in GHD patients [20]. AGHD patients' increased cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α), may stimulate the expression of PAPP-A [21]. What's more, the patient group is somewhat heterogeneous with the majority having pituitary adenomas of different types. Moreover, multiple hormonal deficiencies and different degrees of other

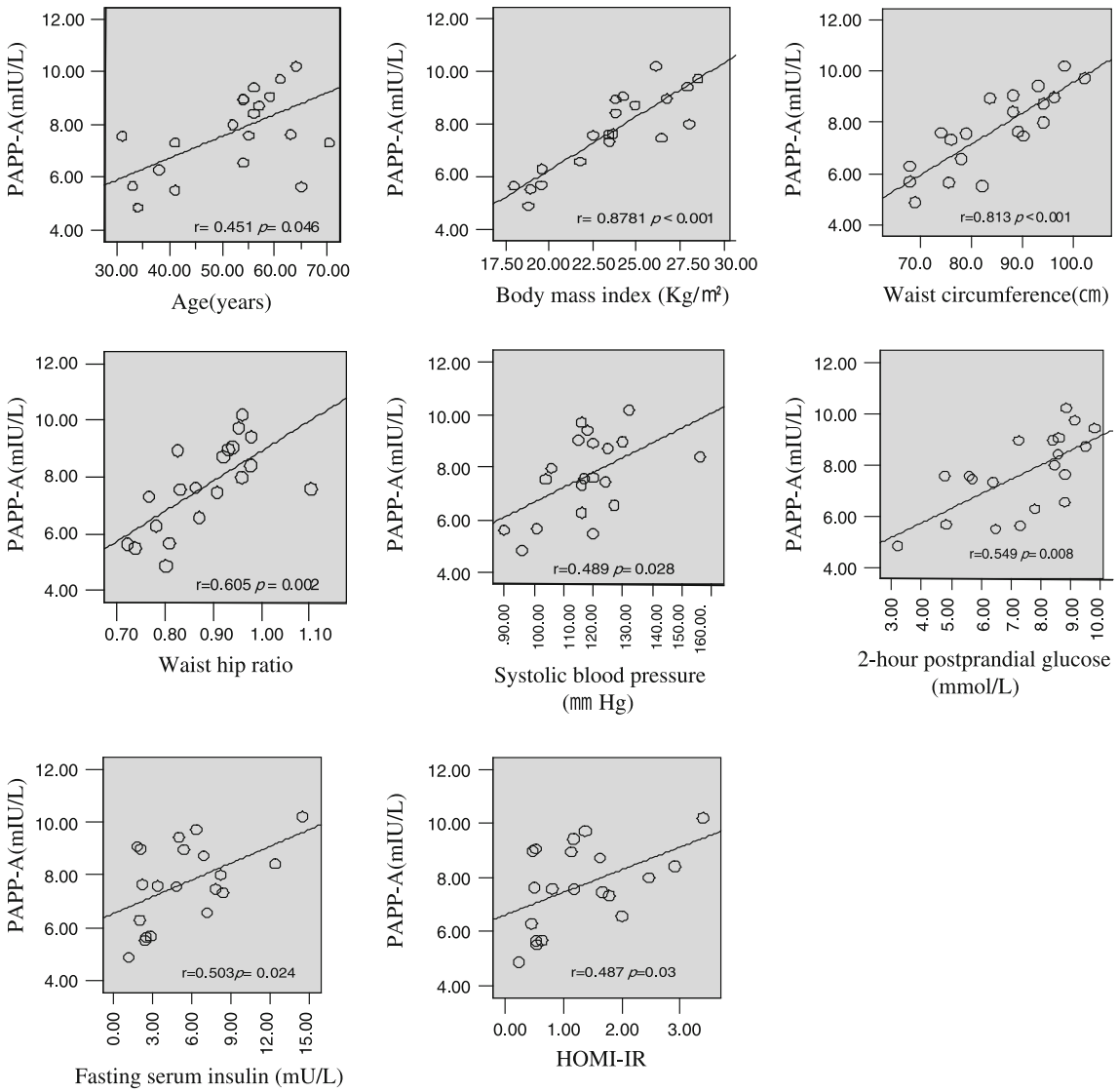

Fig. 1 Correlation between PAPP-A and clinical or biochemical index in AGHD patients. *PAPP-A*: pregnancy-associated plasma protein-A; *HOMA-IR*: Homeostasis model assessment index for insulin resistance

hormonal substitution therapies may also be the confounding factors.

The biological effects of growth hormone are the promotion of growth, stimulation of protein synthesis and lipolysis, and the elevation of blood glucose. AGHD is associated with a shift in the ratio of adipose to muscle tissue, i.e., an increase in adipose tissue and a decrease in muscle [22, 23]. Our study showed that, after matching gender, age, and BMI, the body fat percentage, waist circumference, and waist–hip ratio of the AGHD patients were still higher than those of the healthy control group, indicating that the adipose tissue in AGHD patients had increased, especially the abdominal fat. Studies have shown that obesity is an independent risk factor for atherosclerosis, and the waist–hip ratio is the best predictor. It should be noted that even a low BMI with a high waist–hip ratio is still prognostic of cardiovascular disease. Abdominal obesity is correlated to high morbidity and mortality in patients with cardiovascular diseases [24]. Our study showed that serum PAPP-A was significantly correlated to BMI and waist–hip ratio in the AGHD group. These study results support the belief that serum PAPP-A is indicative of atherosclerosis in AGHD patients, and suggest that serum PAPP-A can be considered as a vascular risk factor in AGHD patients.

Obesity and decreased physical activity that leads to an increase in the ratio of adipose tissue versus muscle in AGHD patients could result in a reduction in insulin sensitivity, even insulin resistance [25]. Arumugam et al. [26] found that the insulin resistance of GHD mouse was accompanied by the dysregulation of pancreatic hormone, adipocytokine secretion, and receptor expression. Our

results showed that serum fasting blood glucose, fasting insulin, HOMA-IR, and HOMA-β in the AGHD group were not significantly different from those of the healthy control group; however, the 2 h postprandial blood glucose was higher in the AGHD group than in the control group. The correlation analysis showed that PAPP-A was correlated to the 2 h postprandial blood glucose, fasting insulin, and HOMA-IR, all of which had substantial effect on the PAPP-A serum level in the AGHD patients. These findings are consistent with those of Ukropec et al. [27]. Their study of 16 AGHD subjects showed that in the absence of growth hormone replacement therapy, serum fasting blood glucose and fasting insulin were not significantly different from those of the healthy control group. However, the 2 h postprandial blood glucose was significantly higher than that of the control group, but was still lower than 7.8 mmol/L. This suggested that postprandial blood glucose in AGHD patients may influenced PAPP-A level. Whether the difference in the postprandial insulin between the two groups led to significant change in postprandial blood glucose is yet to be determined. These results suggested that PAPP-A may be closely correlated to glucose metabolism and insulin resistance in AGHD patients.

The studies in healthy subjects showed that growth hormone was an independent factor for the regulation of total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein in serum [28]. In AGHD research, more attention was focused upon the change in lipid profile than in total cholesterol. Studies showed that triglycerides and low-density lipoprotein significantly increased, whereas high-density lipoprotein significantly decreased in patients without growth hormone replacement therapy [29]. Likewise, our study showed an increase in total cholesterol and triglycerides and a decrease in high-density lipoprotein in AHGD subjects. Triglycerides was correlated to PAPP-A. 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 serum PAPP-A and an alteration in lipid profile suggest a possible link between PAPP-A and atherosclerosis in AGHD patients.

C-reactive protein (CRP), directly contributing to the formation and development of atherosclerosis, is regarded as an independent risk factor for coronary heart disease. Research shows that CRP level is positively correlated to the severity of coronary sclerosis and aortic atherosclerosis [30]. When used as a marker for inflammation, CRP is considered as the strongest predictor for risk of cardiovascular diseases. Although our finding did not show a correlation between CRP and PAPP-A, we observed a significant increase in serum CRP in the AGHD subjects. This alludes to the presence of endogenous inflammation, which is similar to other researchers' observations [31, 32]. The CRP level was increased in AGHD patients without growth hormone replacement therapy and significantly decreased after growth hormone replacement therapy [27, 33]. The changes in the CRP levels in the AGHD patients suggest that chronic inflammation may also play a role in the development of atherosclerosis in this population.

Growth hormone-induced lipolysis enhances the release of FFA from adipose tissue and the increase of FFA concentration in serum. Research showed that there was a decrease in FFA concentration in serum of patients suffered from apituitarism and received routine hormone replacement but not growth hormone replacement [34, 35]. The serum FFA concentration increased after growth hormone replacement therapy was administered [35, 36]. Our study did not find significant difference in FFA concentration between the two groups nor correlation between the PAPP-A and FFA. This could be attributed to the study's small sample size.

Despite using a small sample size, our study was able to show that: (1) Body fat percentage, waist circumference, waist–hip ratio, 2 h postprandial blood glucose, total cholesterol and triglycerides were found to have increased while high-density lipoprotein cholesterol and IGF-1 decreased in AGHD patients. (2) Serum PAPP-A in AGHD patients was positively correlated to BMI, waist circumference, waist–hip ratio, systolic blood pressure, triglycerides, 2 h postprandial blood glucose, fasting insulin, and HOMA-IR. (3) Serum PAPP-A levels were significantly increased in AGHD patients. (4) BMI, 2 h postprandial blood glucose, fasting insulin and HOMA-IR were independent correlative factors of PAPP-A in AGHD patients.

Our findings strongly suggest that the serum PAPP-A levels are related to the atherosclerosis vascular risk factors. However, the size of the study group was not big enough to evaluate the independent effects of the several factors found to be associated with serum PAPP-A in AGHD. Further studies are needed to determine whether the increase in serum PAPP-A is related to the decrease in IGF-1 in AGHD patients and whether reducing the serum levels of PAPP-A can diminish the severity of atherosclerosis and reduce the occurrence of cardiovascular diseases.

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