CLINICAL STUDY

A study of carotid intima-media thickness in GH-deficient Japanese adults during onset among adults and children

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Abstract

Objectives: Increased carotid intima-media thickness (IMT) has been reported among Caucasian adult GH-deficient (AGHD) patients, but not Japanese. Also, it is known that the clinical and biochemical characteristics of AGHD patients are somewhat different based on the onset of the disease in either childhood or adult life. Nevertheless, there has been no study comparing the magnitude of the deviation of their IMT from normal subjects between child-onset (CO) and adult-onset (AO) patients in terms of Z score. The aim of this study, therefore, was first to examine whether Japanese AGHD patients have a risk of early development of atherosclerosis similar to Caucasian patients and secondly to assess the difference in the onset and in progression of atherosclerosis.

Design and subjects: Thirty-four patients (17 CO-AGHD, age 29 ± 7 years, body mass index (BMI) 24 ± 3.8 kg/m² and 17 AO-AGHD, age 48 ± 12 years, BMI 23 ± 3.6 kg/m²) and 34 age- and sex-matched healthy controls (17 CO controls and 17 AO controls) were enrolled in the present study. Blood samples were taken for measurements of lipids, lipoproteins and IGF-I. Subsequently, patients underwent IMT assessment.

Results: CO patients were significantly younger than AO patients. The duration of GH-deficiency in CO patients was significantly longer than that in AO patients. Serum triglyceride (TG) was significantly higher in CO patients than in CO controls ($P < 0.05$). Serum total cholesterol and TG were significantly higher in AO patients than in AO controls ($P < 0.01$). The IMT was significantly greater in CO and AO patients (0.82 ± 0.08 and 0.79 ± 0.03 mm) than in CO and AO controls (0.59 ± 0.02 and 0.68 ± 0.03 mm, $P < 0.01$ and $P < 0.01$ respectively). There was no significant difference in raw values of IMT between CO and AO patients. However, the Z score of IMT calculated using normal Japanese IMT values was significantly higher in CO than in AO patients (2.07 ± 0.68 vs 0.35 ± 0.48 , $P < 0.05$).

Conclusions: These findings suggest that GH deficiency appears to increase an atherosclerotic risk in Japanese AGHD patients, as with Caucasians, and to cause more extensive IMT thickening in CO-AGHD than AO-AGHD patients.

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Introduction

Recent retrospective studies have revealed that adult patients with hypopituitarism under conventional hormone replacement treatment by thyroid hormone, adrenal and/or sex steroids show a higher mortality rate, mostly attributable to vascular disorders, and higher morbidity from diseases related to atherosclerosis than general healthy subjects (1). The early development of atherosclerosis in these patients is thought to be at least partly caused by growth hormone (GH) deficiency. The severity of atherosclerosis has been assessed by measurement of carotid intima-media thickness (IMT), a well-accepted marker of vascular

risks, by means of high resolution ultrasonography. IMT was found to increase in adult Caucasian patients with hypopituitarism (2) as well as child-onset (CO) adult GH-deficient (AGHD) patients (3). Furthermore, a 1 year GH treatment of AGHD patients resulted in a significant amelioration of their increased IMT (4).

GH-deficient (GHD) patients also have associated abnormal metabolism of lipid and carbohydrate, which may contribute to an increased risk of vascular disease (5–13). Administration of GH to these patients reduced adipose tissue and increased lean body mass (14, 15), increased physical and cardiac performance (16–19), normalized lipid metabolism (9) and improved quality of life (20, 21). However, AGHD may not be a

homogeneous clinical entity because clinical characteristics of AGHD appear to be somewhat different between CO and adult-onset (AO) patients. Attanasio *et al.* (22) reported that the body height, weight, body mass index (BMI), lean body mass and the waist/hip ratio of AO patients were all significantly greater than in CO patients but there were no differences in serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels between the two groups. We wondered whether the atherosclerotic risk is similar among AO and CO patients. To the best of our knowledge, there is no report comparing the Z score of IMT among AO and CO patients so far. Furthermore, IMT in Japanese AGHD patients remains not definitively studied.

The aim of this study was to assess the progression of atherosclerosis by means of IMT measurement in Japanese AGHD patients and to analyse how it differs in a comparison between Japanese CO and AO patients.

Patients and methods

Patients

Thirty-four patients (17 CO, 10 males and 7 females, age 29 ± 7 years, height 1.62 ± 0.02 m, BMI 24 ± 3.8 kg/m² and 17 AO, 10 males and 7 females, age 48 ± 12 years, height 1.61 ± 0.02 m, BMI 23 ± 3.6 kg/m²) and

34 healthy controls (17 CO controls and 17 AO controls), comparable in gender and age distribution were enrolled in the present study (Table 1). The aetiology of hypopituitarism was varied among the patients as shown in Table 1. In all patients, GH deficiency was diagnosed as peak serum GH levels below 3 µg/l in insulin tolerance tests.

All CO patients were treated with GH at the dose of 0.5 IU/kg body weight per week for a period ranging from 1 to 11 years, but GH administration discontinued at least 3 years before entry into the study, whereas none of the AO patients had been given GH therapy (Table 1). All patients had multiple pituitary deficiency and were under replacement therapy with various hormones such as thyroxine, hydrocortisone or desmopressin at standard doses. All patients with hypogonadism below age 50 except four CO and two AO patients had sex hormone replacement. No medications other than hormones were prescribed. Patients known to have diabetes, cardiovascular diseases or hypertension were excluded. All participants gave their informed consent, and the protocol was approved by the local institutional review board in our hospital.

Protocol

All participants were studied in the postabsorptive state after a 12 h overnight fast. Blood pressure (systolic and

Table 1 Characteristics of the study population. Values are means $\pm$ s.d.

	CO controls	CO-AGHD	AO controls	AO-AGHD
No.	17	17	17	17
Age (years)	29 ± 7	29 ± 7^b	48 ± 12	48 ± 12
Sex (M/F)	10/7	10/7	10/7	10/7
Height (m)	1.67 ± 0.07	1.62 ± 0.08^a	1.64 ± 0.02	1.61 ± 0.02^c
BMI (kg/m ²)	22 ± 1.6	24 ± 3.8^a	22 ± 3.2	23 ± 3.6
Smoking	2/17	2/17	2/17	2/17
Duration of GHD	—	21 ± 9^b	—	14 ± 9
Duration of GH treatment	—	7 ± 4	—	0
PAS (mmHg)	109 ± 15	108 ± 15	115 ± 7	111 ± 11
PAD (mmHg)	62 ± 9	71 ± 11	73 ± 9	72 ± 12
Aetiology (no.)				
Pituitary adenoma	—	1	—	12
Craniopharyngioma	—	3	—	1
Germinoma	—	5	—	0
Optic glioma	—	1	—	0
Sheehan's syndrome	—	0	—	1
Trauma	—	1	—	2
Idiopathic	—	6	—	1
Replacement treatment				
Thyroxine	—	15	—	14
Cortisol	—	14	—	14
Sex steroids	—	9	—	7
Desmopressin	—	6	—	1
TC (mg/dl)	188 ± 15	211 ± 48	186 ± 16	209 ± 24^c
LDL-C (mg/dl)	109 ± 14	126 ± 35	111 ± 14	119 ± 19
HDL-C (mg/dl)	60 ± 9	55 ± 18	59 ± 10	61 ± 24
Total TG (mg/dl)	58.8 ± 27.6	159 ± 121^a	78.3 ± 36.2	143 ± 79^c
IGF-I (ng/ml)	—	63 ± 35	—	60 ± 24
Fasting glucose (mg/dl)	81 ± 12	80 ± 13	82 ± 9	80 ± 10

^a $P < 0.05$ vs CO control, ^b $P < 0.01$ vs AO-AGHD, ^c $P < 0.01$ vs AO control. PAS, systolic blood pressure; PAD, diastolic blood pressure.

diastolic) was measured using the sphygmomanometric method. Blood samples were taken for measurements of lipids, lipoproteins and insulin-like growth factor-I (IGF-I). Then, patients underwent ultrasonographic scanning of the carotid arteries according to a recent Japanese study (24) by a trained physician and were photographed. The assessment of IMT was performed by a high resolution echo-colour Doppler system (LOGIQ700MR; GE Yokogawa Medical System, Tokyo, Japan). Scanning of the extracranial carotid arteries in the neck was performed bilaterally in three different longitudinal projections: anterior-oblique, lateral and posterior-oblique as well as the transverse projection. Three determinations of intima plus medial thickness were conducted at the site of the greatest thickness and at two points, 1 cm upstream and 1 cm downstream from the site of the greatest thickness. These three values were averaged. The greatest value among the six averaged intima plus medial thickness (three from the left and three from the right) was used as the representative IMT value for each individual. The coefficients of variation of the measurements were less than 3%. All scans were read by an independent physician, blinded as to the clinical status of the subjects. This method is well-known and valid in Japan, although performed in a different way from most previous investigation of IMT in GHD patients. The Z score of IMT was defined as the standard deviation of mean IMT values in normal Japanese of every 10 year age cohort (23).

Serum TC, HDL-C, serum triglyceride (TG) and plasma glucose levels were assayed by standard laboratory techniques. LDL-C was estimated by the Friedewald equation. Serum IGF-I levels were determined by an IRMA after extraction (SRL, Inc., Tokyo, Japan).

Statistical analysis

Data are expressed as means $\pm$ S.E.M. in Figures and means $\pm$ S.D. in the Table. Statistical analysis was performed using one-way repeated measures ANOVA in both the Table and Fig. 1, and using Student's *t*-test in Fig. 2. $P < 0.05$ was considered significant.

Results

The comparison of the background data for the CO and AO patients revealed the age and the duration of GH deficiency to be significantly different among the two groups. The average age of CO patients was significantly younger than that of AO patients ($P < 0.001$), and the duration of GH deficiency in CO patients was significantly longer than in AO patients ($P < 0.01$). However, all CO patients had a past history of GH treatment to ameliorate their short stature. The duration of GH administration was varied among patients. The spectrum of causes of GH deficiency was entirely different

Figure 1 Carotid IMT in CO-AGHD, CO control, AO-AGHD and AO control. The values represent the means $\pm$ S.E.M. * $P < 0.01$ vs CO control; ** $P < 0.01$ vs AO control.

between CO and AO patients. The prevalence of cigarette smoking, blood pressure and hormone replacement were similar among the CO and AO patients (Table 1).

Furthermore, compared with the data of control subjects, height was significantly shorter in CO patients than in CO controls and was significantly shorter in AO patients than in AO controls ($P < 0.05$). BMI was significantly higher in CO patients than CO controls ($P < 0.01$), but not significantly different among AO patients and AO controls. Serum TC, LDL-C, HDL-C and glucose were similar in CO patients and CO controls. Serum TG was significantly higher in CO patients than in CO controls ($P < 0.05$). Serum LDL-C, HDL-C and glucose were similar in AO patients and AO controls. Serum TC and TG were significantly higher in AO patients than in AO controls ($P < 0.01$ and $P < 0.01$ respectively). However, no difference was observed in serum TC, LDL-C, HDL-C, TG, IGF-I and glucose and in BMI among CO and AO patients (Table 1).

The IMT in AGHD patients was significantly greater than that in control subjects irrespective of the onset of their GH deficiency (CO patients, 0.82 ± 0.08 mm vs CO controls, 0.59 ± 0.02 mm, $P < 0.01$; AO patients, 0.79 ± 0.03 mm vs AO controls, 0.68 ± 0.03 mm, $P < 0.01$) (Fig. 1). Height and BMI are major determinants of IMT. CO and AO patients were significantly shorter in height in comparison with the respective controls (Table 1). Therefore, IMT was corrected by height. IMT/height was still significantly greater in CO patients than in CO controls (0.508 ± 0.20 vs 0.365 ± 0.064 , $P < 0.01$). IMT/height was also significantly greater in AO patients than in AO controls (0.491 ± 0.028 vs 0.425 ± 0.021 , $P < 0.05$). Since BMI was significantly higher in CO patients than CO controls, IMT was corrected by BMI in CO patients

Figure 2 Comparison of the Z score of IMT in CO-AGHD and AO-AGHD. The values represent the means $\pm$ S.E.M. * $P < 0.05$ vs AO-AGHD.

and CO controls. IMT/BMI was still significantly higher in CO patients than CO controls (0.036 ± 0.016 vs 0.029 ± 0.007 , $P < 0.05$). There was no significant difference in absolute values of IMT among CO and AO patients. However, the IMT increases as a variable dependent on age in normal subjects, so the Z score of the IMT was calculated in each patient. The Z score of IMT was significantly higher in CO patients than in AO patients (2.07 ± 0.68 vs 0.35 ± 0.48 , $P < 0.05$) (Fig. 2).

Discussion

It is well known that GH deficiency causes increased body fat with a decrease in lean body mass and abnormal levels of serum lipids and lipoproteins irrespective of racial difference (24). In addition, increased insulin resistance in peripheral tissues, decreased fibrinolytic

activities, abnormal cardiac structure and performance, and premature atherosclerosis with increased arterial IMT have been reported in Caucasian patients with GH deficiency (1–3, 7, 13, 16), all of which might be responsible for an increased incidence of cardiovascular morbidity and mortality (1).

Asian people, including Japanese, have their own customs and conventions, different from Caucasians', particularly preference for food, and furthermore, the body constitution of Asian people is also different from that of Caucasian people, probably due to differences in genetic and environmental backgrounds. Therefore, it is of considerable interest whether Asian patients with GH deficiency are exposed to the risk of premature atherosclerosis in the same way as Caucasian patients. There are no convincing reports regarding the morbidity and mortality in Asian patients with GH deficiency.

To assess the development of premature atherosclerosis, we decided to measure the IMT of common carotid arteries, since an increased IMT is known to be the most sensitive parameter of atherosclerotic changes and to be detected without obvious abnormalities of the classic vascular risk factors (23, 25–27). In this study, we found that the IMT of the common carotid arteries was significantly increased in AGHD irrespective of timing of the disease onset. Our findings were not completely consistent with the findings of the pioneer study in Caucasian patients by Markussis *et al.* (2). The IMT in our AO-AGHD patients was 0.79 mm on the average, which was comparable to 0.72 mm, the value in their patients aged 40–60 years. In contrast, the IMT, 0.82 mm on the average, in our CO-AGHD patients aged 29 ± 7 years seems to be unexpectedly great since the IMT was 0.50 mm among the patients less than 40 years of age in Markussis' study. Besides, our IMT data of CO-AGHD patients were consistent with a more recent report by Capaldo *et al.* (3) that the IMT was as great as 0.83 mm in the CO-AGHD aged 25 ± 1 years, comparable to that in our patients. These discrepancies may simply be caused by the difference among methods for IMT measurement. Our method is, of course, a valid (23) and previously reported method.

On the other hand, there is no report comparing the IMT of AGHD patients of CO and AO in one study. As mentioned above, our CO- and AO-AGHD patients showed greater IMT of the carotid arteries than the respective control subjects. There was no significant difference in the absolute values of IMT between CO and AO patients. This finding appeared to be unusual since the carotid artery IMT would be age-dependently increased in normal subjects. Then we further calculated the Z score of the IMT in each AGHD patients using the normal Japanese IMT values of every 10 year age cohort (23). It is of interest that the Z score of IMT was significantly higher in CO patients than

AO patients, suggesting that CO patients have a greater risk of early development of atherosclerosis.

The reason why premature atherosclerosis is more prevalent in CO patients continues to be unclear. The simple explanation would be that these findings are due to the longer duration of GH deficiency. Indeed, the duration of GH deficiency in our CO patients was significantly longer than that in AO patients.

Borson-Chazot *et al.* (4) reported that a 1-year GH treatment resulted in significant reduction in increased IMT of carotid arteries in GHD patients, giving strong evidence that GH itself affects carotid IMT. Their findings also indicate that carotid IMT is not completely fixed but rather reversible and changeable under certain circumstances. Carotid IMT is affected by many factors including serum lipids. In our study, serum lipid levels in CO patients tended to be higher than those in AO patients, although the difference was statistically not significant (Table 1). Furthermore, it is well known that nitric oxide (NO) is a mediator of vasodilatation, inhibition of platelet aggregation, leukocyte adhesion and inhibition of vascular smooth muscle cell growth. IGF-I induces NO in vascular endothelial cells and NO may mediate haemodynamic effects of recombinant GH in GHD patients (28, 29). In this study, IGF-I was really low in AGHD although we did not measure IGF-I levels in normal subjects who were of normal height. We thought that GHD patients indeed would have lower plasma IGF-I levels as well as NO production than normal subjects. Although IGF-I levels did not differ between CO and AO patients (Table 1), CO patients were significantly younger than AO patients. Since it is well known that IGF-I levels progressively decline with increasing age, it would be biologically plausible that IGF-I levels in CO patients were markedly lower than in AO patients. Indeed, this difference could explain why CO patients had a higher Z score of IMT than AO patients. Hence, an enhanced effect through the combination of GHD as well as lower IGF-I levels for age and lipid abnormalities may simply be attributed to the greater Z score of IMT in CO patients.

In summary, this study demonstrated for the first time that carotid IMT in Japanese AGHD patients is increased as much as in Caucasian patients. Furthermore, Japanese CO-AGHD patients showed greater IMT than AO-AGHD patients, although the reason remains to be clarified.

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