Elevation of E-Selectin Concentrations may Correlate with Potential Endothelial Dysfunction in Individuals with Hypopituitarism During Therapy with Growth Hormone

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Abstract: Increased mortality due to cardiovascular disease has been described in adult patients with untreated growth hormone (GH) deficiency. GH replacement therapy has been demonstrate to improve vascular reactivity and reverses early atherosclerotic changes in GH deficient adults. The objective of this study was the assessment of fibrinolytic markers, soluble adhesion molecules, inflammatory cytokines and endothelial function in hypopituitary adults with GH deficiency and with GH replacement therapy. We studied 20 GH deficient patients, 10 men and 10 women (aged, 43.4 ± 8.4 years) under GH replacement therapy compared with a control group matched for age and body mass index, 9 men and 16 women. All subjects, patients and controls, were life-long non-smokers, normotensive and non-diabetic. The following variables were recorded: anthropometrical and body composition variables, serum concentrations of glucose, insulin and C-peptide; thrombin anti-thrombin fragments and fibrin degradation product D-dimer that were determined by an enzyme-linked-immunosorbent assay (ELISA); IGF-I by radioimmunoassay; C-reactive protein by highly sensitive immunonephelometry; E-selectine, P-selectine, soluble intercellular cell adhesion molecule-1, soluble vascular cell adhesion molecule-1, interleukin-6 and monocyte chemoattractant protein-1 by ELISA. The assessment of endothelial function *in vivo* was measured by Doppler. Patients with GH deficiency had higher hip/waist ratio and C-peptide and triglycerides concentrations than controls. Our results demonstrated no difference in fibrinolytic markers among patients and controls. E-selectin concentrations were higher in patients than in controls, 22.5±11.4 vs. 10.7±6.2 μg/L, p= 0.0001. P-selectin, soluble intercellular cell adhesion molecule-1, soluble vascular cell adhesion molecule-1, interleukin-6, monocyte chemoattractant protein-1 and C-reactive protein were similar in the 2 groups. Vascular reactivity and carotid intima-media thickness were also similar in patients and controls. In this study we have demonstrated in adults with GH deficiency under GH substitution elevation of E-selectin concentrations that may correlate with potential endothelial dysfunction suggesting that the protective effect of GH in these patients may be enhancing other mechanisms.

Key Words: Endothelial dysfunction, growth hormone, growth hormone deficiency, C-reactive protein, insulin growth factor-I, monocyte chemoattractant protein-1, E-Selectin, P-Selectin.

INTRODUCTION

Increased cardiovascular mortality and carotid atherosclerosis have been observed in hypopituitary adult patients with untreated growth hormone deficiency (GHD) due to cardiovascular disease compared with age-matched controls (De Boer *et al.,* 1995; Tomilnson *et al.,* 2001; Feldt-Rasmussen *et al.,* 2004). Several cardiovascular risk factors are present in GHD patients and it is well established that GHD is associated with abnormalities in body composition, especially in body mass index (BMI) that may have an impact on cardiovascular risk factors and also it has been observed that GHD is linked to endothelial dysfunction (Leonsson *et al.,* 2002; Smith *et al.,* 2003; Klibanski, 2003; Lanes *et al.,* 2005). On the other hand, atherogenesis begins long before the presence of clinically detectable disease and endothelial dysfunction is an early and potentially reversible event in the pathogenesis of atherogenesis and some studies showed that earyl-onset carotid atherosclerosis is associated with increased intima-media thickness and elevated serum levels of inflammatory markers (Pfeizer *et al.* 1999; Klibanski, 2003; Elhadd *et al.,* 2001; Magyar *et al.,* 2003). Recent studies have shown that inflammation plays a critical role in pathogenesis of atherosclerosis and cardiovascular risk and reports by many investigators have suggested a number of effects that GH may have on inflammatory cells (Pfeizer *et al.* 1999; Leonsson *et al.,* 2003; Feldt-Rasmussen *et al.,* 2004; Lanes *et al.,* 2005; McCallum *et al.,* 2005). Extravasation of white blood cells is an important element in atherogenesis and the endothelium orchestrates this recruitment of leukocytes that is initiated by members of selectin gene families of soluble adhesion molecules, which are expressed on the luminal surface of vascular cells (Elhadd *et al.,* 2001). Currently one can examine biophysical and biochemical markers of endothelial dysfunction also including endothelial

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function *in vivo*, peripheral fibrinolytic markers, soluble adhesion molecules and inflammatory cytokines (Celermajer *et al.,* 1992; Gómez *et al.,* 2006).

Also GH replacement has been demonstrate that improves vascular reactivity, regresses carotid intima-media thickness and reverses early atherosclerotic changes in GHD adults, and the question is whether GH, or its downstream mediator insulin-growth factor-I (IGF-I), modulate the level and degree of endothelial activity (Celermajer *et al.,* 1992; Colao *et al.,* 2002; Klibanski, 2003; Abdu *et al.,* 2004; Gómez *et al.,* 2006). On the other hand some studies have demonstrated that GH replacement in GHD has beneficial effects on peripheral markers of inflammatory activity or on soluble adhesion molecules (Pfeizer *et al.* 1999; McCallum *et al.,* 2005).

The aims of this study were to investigate the differences in biochemical parameters, fibrinolytic markers, soluble adhesion molecules, peripheral inflammatory cytokines and endothelial function biophysically studied, in GHD patients chronically treated with GH replacement therapy and in controls matched for age and BMI.

MATERIALS AND METHODS

Assessment of Patients and Controls Characteristics

Twenty patients who had adult-onset GHD, 10 men and 10 women, aged 43.4 ± 8.4 years, due to non-functioning pituitary adenoma (n= 10), craneopharyngioma (n= 4), idiopathic hypopituitarism (n= 2) and Sheehan´s syndrome (n= 4) (Treated group), were studied. This Treated group was compared with a control group matched for age and BMI, 9 men and 16 women (aged, 40.9 ± 9.1 years) (Control group). For all subjects, patients and controls, the age was between 30 and 55 years, were life-long non-smokers, more than 10 years, normotensive and non-diabetic, with normal resting electrocardiogram and without family history of diabetes mellitus, premature vascular disease, or chronic diseases, and without pharmacological treatment or other discernible risk factors for the development of endothelial dysfunction and BMI was between 22 and 32 Kg/m^2.

In all cases, GHD patients and controls, we studied the anthropometric and body composition variables, biochemical and fibrinolytic markers, soluble adhesion molecules, peripheral inflammatory cytokines and endothelial function.

GHD patients (Treated group) had a full history taken and a physical examination; hormonal evaluation included the study of GH, thyrotropin, adrenocorticotropin, gonadal axis and arginine-vasopressin secretion in all cases. The age of onset of the pituitary disorder and the diagnosis of GHD was 36 ± 6.1 years and the time from disease to study start was 7.1 ± 6.7 years and the number of additional deficiencies were 2.5 ± 1.1. All patients were receiving standard hormone replacement therapy, for any combination of secondary adrenal, gonadal and thyroid failure as well as diabetes insipidus. All GHD patients were chronically treated with GH substitution and was initiated 4.1 ± 1.8 years before and all the patients were treated with GH using an automated pen-injection device (KabiPen® or Genotropin Pen®, Pfizer). The starting dose was 0.24 ± 0.13 mg/day once daily and the final dose was 0.42 ± 0.11 mg/day; the criteria used to determine whether the GHD patients received adequate GH replacement was IGF-I concentrations between normal range for sex and age according of our control data (Gómez *et al.,* 2003).

BMI was calculated as body weight divided by height squared (kg/m^2), waist-hip ratio was determined and blood pressure was measured in the supine position after 5 minutes of rest. Body composition was determined by a bioelectrical impedance analyser using a formula provided by the manufacturers (Holtain BC Analyser, UK) (Kyle *et al.,* 2004). The results obtained were: total water in L, free fat mass in Kg, fat mass in Kg, and body fat in percentage.

The following variables were recorded in Treated group and Control group: age, body weight, BMI, blood pressure, waist and hip measurements, waist/hip ratio, body composition parameters, number of additional pituitary hormone deficiencies, and in all cases after an overnight fast, blood was obtained for measurement of serum concentrations of glucose, basal insulin, C-peptide, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, fibrinogen, thrombin anti-thrombin fragment (TAT) and fibrin degradation product D-dimer, IGF-I, E-selectin, P-selectin, soluble intercellular cell adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), interleukine-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and C-reactive protein (CRP).

Blood samples were obtained in the morning (0800-0900), and serum was frozen at –80ºC until analysis.

Diabetes mellitus and glucose intolerance were ruled out in patients and controls; after a 12-h overnight fast, glucose was ingested in a dose of 75 g, and blood samples were collected at 0, 30, 60, 90 and 120 minutes.

The insulin tolerance test was used to diagnose GHD in all patients and as other GH stimulation test was used glucagon stimulus in all patients (Gómez *et al.,* 2002). The criterion for entry of patients into the study was a GH peak response of less than 3 μg/L after the insulin tolerance test and after glucagon stimulus.

All patients and controls gave their informed consent for the study, which was approved by the ethics committee at the hospital.

Haemodynamic Measurements

Throughout the period of measurements, the subject rested supine in a climatized room (22ºC) and the system comprises a Doppler continuous wave with a 8-MHz linear phased-array transducer to determine systolic blood pressure in both brachial, humeral and posterior tibial arteries. For intima-media thickness assessment examination was performed in common carotid and femoral with the 8-MHz linear phased-array transducer; the images were measured in an automated analysing system and the intima-media thickness was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall. The mean of 2 separately analysed images from each vessel area was used in the statistical analysis.

The assessment of endothelial function *in vivo* was measured by Doppler (Ultramark 9 HDI, Advances Technology Laboratories, Bothel, USA) and with a linear phased-array transducer giving high axial resolution. The brachial artery was identified using the ultrasound transducer and after 10 minutes resting a two-dimensional longitudinal M-mode image of the brachial artery was obtained simultaneously with R wave of the ECG. Blood artery flow was calculated by multiplying the mean blood velocity corrected for Doppler angle by the internal brachial artery diameter measured by wall tracking. Measurements were made at baseline and after a sphygmomanometer was inflated at the wrist to suprasystolic pressure (250 mmHg for 5 minutes). Blood flow was recorded from 30 seconds after cuff release, and internal artery brachial diameter was measured at 90 seconds. All measurements were repeated 10 minutes later and 3 minutes after the administration of sublingual trinitrate (400 μg), an endothelium-independent vasodilator; the difference between the 2 determinations was the non-endothelial vasodilatation dependent (Celermajer *et al.,* 1992).

Assays

Plasma glucose was measured using glucose analyser (Hitachi autoanalyser). Serum insulin and C-peptide with enzymochemoluminiscence assay in solid phase (Medgenix Diagnostics) and (Daichii Laboratories), respectively; the inter-assay and intra-assay coefficient of variation were 7.3% and 6% for insulin and 12.8% and 4.2% for C-peptide. Insulin resistance was calculated through the homeostasis model assessment index (HOMA) from fasting plasma glucose and insulin concentrations (Matthews *et al.,* 1985). Total serum cholesterol was measured through the reaction of cholesterol esterase / cholesterol oxidase / peroxidase; HDL - cholesterol after precipitation with polyethylene glycol at room temperature; total serum triglycerides through the reaction of glycerol-phosphate-oxidase and peroxidase and LDL-cholesterol concentrations were calculated from total serum cholesterol and HDL-cholesterol.

Fibrinogen was measured by the functional method (IL test fibrinogen C, Instrumentation Laboratory, Milan, Italy); TAT fragments by enzyme-linked immunosorbent assay (ELISA) (Enzygnost TAT micro, Dade Behring, Germany) and the fibrin degradation product D-dimer with an ELISA method (Enzygnost D-Dimer micro, Dade Behring Marburg, Germany); the inter-assay and intra-assay coefficient of variation were 15% and 6% for TAT fragments, and 10% and 5% for and the fibrin degradation product D-dimer. Serum concentrations of IGF-I were determined by radioimmunoassay after separation of IGFs from IGF-binding proteins by acid–ethanol extraction and with des(1–3)-IGF-I as radioligand, to minimise interference of IGFBPs in the extract; the intra-and inter-assay coefficients of variation were 10% and 3.1%, respectively. MCP-1 and IL-6 levels were measured by ELISA (R&D Systems); plasma CRP protein by a highly sensitive immunonephelometry kit (Dade Behring, Marburg, Germany); E-selectin, P-selectin, ICAM-1 and VCAM-1 by ELISA (Bender Medsystems, Kits BMS205, BMS192, BMS201 and BMS232, respectively). To define the specificity of these determinations several polypeptides were tested and there were no cross-reactivity determined for any of the tested proteins and no interference with other members of the immunoglobulin family.

Statistical Analysis

Usual statistics (mean and standard deviation) have been used to describe the data and the Kolmogorov-Smirnov test was applied to check the normality of the variables. The ANOVA test was used to compare quantitative data among - groups for independent samples. Relationships among variables were sought by Pearson´s correlation coefficient. If the possibility of chance occurrence was $p < 0.05$, it was considered statistically significant (Rossner, 1995). All statistical analysis was performed using the Statistical Package for Social Sciences (SPSS/Windows version 8.0, SPSS inc., Chicago IL, USA).

RESULTS

The background characteristics of the study populations in Treated group and Control group are shown in Table **1**. Patients with GHD and with GH substitution (Treated group) had higher hip/waist ratio, 0.92±0.04 vs. 0.86±0.08, p= 0.012, basal C-peptide 1.03±0.44 vs. 0.68±0.18 nmol/L, p= 0.005, than those in Control group, without differences in HOMA index; triglycerides concentrations, 1.6±0.3 vs. 0.83±0.29 mmol/L, p= 0.001, were also higher in Treated group than in Control group. Our results demonstrated no difference of fibrinogen, TAT and fibrin degradation product D-dimer concentrations between the 2 groups. IGF-I concentrations were similar in the 2 Groups (Table **2**). E-selectin concentrations were higher in Treated group than in Control group, 22.5±11.4 vs. 10.7±6.2 μg/L, p= 0.0001. P-selectin, ICAM-1, VCAM-1, IL-6, MCP-1 and CRP were similar in the 2 groups (Table **3**). Brachial dilatation mediated by endothelium, brachial dilatation non-mediated by endothelium, right brachial vessel calibre intima-media thickness, left brachial vessel calibre intima-media thickness, were also similar in the 2 groups (Table **4**) (Fig. **1** and **2**).

In the Treated group, we found a positive correlation between E-selectin and ICAM-1 (r= 0.34, p= 0.001), E-selectin and MCP-1 (r= 0.36, p= 0.001), IGF-I and the fibrin degradation product D-dimer (r= 0.44, p= 0.03) and an inverse correlation between IGF-I and MCP-1 (r= -0.41, p= 0.03), without correlation with the number of additional pituitary hormone deficiencies, and the fibrinolytic markers, endothelial-mediated vasodilatation response and intima-media thickness.

DISCUSSION

Adults GHD is known to be associated with reduced life expectancy related to excess vascular events and several cardiovascular risk factors are present (De Boer *et al.,* 1995; Tomilnson *et al.,* 2001) and in women are particularly increased the risk for coronary artery disease and stroke (Leonsson *et al.,* 2002; Smith *et al.,* 2003; Klibanski, 2003; Lanes *et al.,* 2005; Kelestimur *et al.,* 2005). Endothelial dysfunction is also present in patients with this condition which GH replacement reverses (Celermajer *et al.,* 1992; Colao *et al.,* 2002; Abdu *et al.,* 2004). Increasing evidence suggests that GHD shows features of the common inflammatory basis

Table 1. Anthropometrical, Blood Pressure and Body Composition Characteristics of Patients and Controls. Treated Group: Patients with GH Deficiency and with Chronic GH Substitution. Control Group: Controls. BMI: Body Mass Index. Data are Expressed as Mean ± Standard Deviation and in Parentheses Range

	Treated Group	Control Group	Difference (p)
Age	43.4 ± 8.4 (30-53)	40.9 ± 9.1 (30-55)	0.41
BMI (Kg/m^2)	28.7 ± 5.6	26.0±3.9	0.51
Hip/waist ratio	0.92 ± 0.04	0.86±0.08	0.012
Systolic blood pressure (mm Hg)	120.1 ± 24.1	111.6±12.9	0.45
Diastolic blood pressure (mm Hg)	72.4 ±15.6	70.5±9	0.41
Total body water (L)	38.1±11	38.0±7.5	0.51
Fat free mass (kg)	52.1±15.1	52.0±10	0.503
Fat mass (Kg)	18.6±6.3	15.5±8.3	0.182
Body fat (%)	25.9±4.4	26.1±10.6	0.12

Table 2. Biochemical Characteristics, Homeostasis Model Assessment (HOMA) and Coagulation Parameters of Patients and Controls. Treated Group: Patients with GH Deficiency and with GH Substitution. Control Group: Controls. TAT: Thrombin Anti-Thrombin Fragment. D-Dimer: Fibrin Degradation Product D-Dimer. IGF-I: Insulin-Growth Factor-I. Data are Expressed as Mean ± Standard Deviation

	Treated Group	Control Group	Difference (p)
Glucose (mmol/L)	5±0.6	5.1±0.6	0.49
Basal insuline (pmol/L)	80.4±41.1	65.1±23.3	0.08
Basal C-peptide (nmol/L)	1.03±0.39	0.68±0.18	0.015
HOMA	2.6±1.4	2.09±0.8	0.5
Total cholesterol (mmol/L)	5.2±0.5	5±0.9	0.15
HDL cholesterol (mmol/L)	1.6±0.1	1.51±0.28	0.12
LDL cholesterol (mmol/L)	2.9±0.5	3.08±0.83	0.288
Triglycerides (mmol/L)	1.6±0.3	0.83±0.29	0.001
Fibrinogen (g/L)	3.1±0.5	3±0.65	0.67
TAT (μg/L)	17.5±13.6	15.3±6.74	0.308
D-dimer (μg/L)	218.9±34	238.32±84.5	0.118
IGF-I (nmol/L)	39.4±10.2	42.4±12.4	0.32

and a considerable body of evidence supports the notion that various mediators such as cytokines are involved in the process of atherosclerotic lesions (Ross, 1999; Wellen *et al.*, 2005). Recent studies show that early-onset carotid atherosclerosis is associated with increased intima-media thickness and elevated serum levels for inflammatory markers (Ross, 1999; Linton and Fazio, 2003; McCallum *et al.*, 2005; Wellen *et al.*, 2005). Some studies confirm significant endothelial dysfunction in hypopituitarism and provide insight into the relationship between biochemical and biophysical markers of early atherosclerosis in GHD patients (Elhadd *et al.*, 2001) and after GH therapy abnormalities of endothelial activation improved (Böger *et al.*, 1996; Kvaniscka *et al.*, 2000; Abdu *et al.*, 2004; Silha *et al.*, 2005; Yuen *et al.*, 2005). However the studies are controversial and some of them demonstrated that endothelium-dependent vasodilatation is intact in GH treated GHD patients with the persistence of a prothrombotic tendency and endothelial dysfunction after the GH treatment (Smith *et al.*, 2003). Other results indicate that high BMI in GHD patients contribute to their

Table 3. Endothelial and Inflammatory Parameters in Patients and Controls. Treated Group: Patients with GH Deficiency and with GH Substitution. Control Group: Controls. ICAM-1: Intracellular Adhesion Molecule-1, VCAM-1: Vascular Adhesion Molecule 1, IL-6: Interleukine 6 and MCP-1: Monocyte Chemoattractant Protein-1

	Treated Group	Control Group	Difference (p)
E-selectin (μg/L)	22.5±11.4	10.7±6.2	0.0001
P-selectin (μg/L)	70.4±13.5	67.4±42.6	0.45
ICAM-1 (μg/L)	211.1±51.3	197.4±51.3	0.083
VCAM-1 (μg/L)	652.3±162.1	573.6±121.2	0.572
IL-6 (ng/L)	3.5±0.9	3.79±1.42	0.87
MCP-1 (ng/L)	181.2±44.3	151.9±40.8	0.186
C-reactive protein (mg/L)	2.3±1.1	1.56±1.5	0.423

Table 4. Endothelial Data Obtained Following High-Resolution Studies of the Brachial Arteries in Patients and Controls. Treated Group: Patients with GH Deficiency and with GH Substitution. Control Group: Controls

	Treated Group	Control Group	Difference (p)
Brachial dilatation mediated by endothelium (%)	4.81±0.7	4.75±5.14	0.944
Brachial dilatation non-mediated by endothelium(%)	14.3±2.3	16.1±2.1	0.265
Right intima-media thickness (mm)	0.6±0.21	0.69±0.33	0.87
Left intima-media thickness (mm)	0.52±0.1	0.42±0.84	0.586

increased intima-media thickness (Smith *et al.,* 2003). Other studies show that biophysical test of endothelial function improved after 1 year of GH substitution but without significant change in biochemical endothelial or inflammatory markers and with a decrease in coronary risk mainly due to reduction in systolic and diastolic blood pressure and increase in HDL-cholesterol (Leonsson *et al.,* 2002).

E-selectin and P-selectin are only found on activated endothelium in contrast to ICAM-1, VCAM-1 who are related molecules belonging to the immunoglobulin supergene family and are ligands for leukocyte integrins and are thought to stabilize the adhesion of leukocytes to endothelium and to be involved in cellular interactions within tissues. P-selectin, ICAM-1 and VCAM-1 are expressed in endothelial cells but has also been demonstrated on lymphocytes and other cells (Linton and Fazio, 2003) as plasmatic cells (Elhadd *et al.,* 2004). Elevated concentrations of these factors have been described in cardiovascular diseases and several studies of the biomarkers of endothelial dysfunction have been done in patients with diabetes mellitus or insulinresistance (Blann and Lip, 2000). Although most of them have demonstrated increased concentrations of E-selectin, P-selectin, ICAM-1 and VCAM-1, there are a broad heterogeneity in the results mainly in which of these factors is more alterated (Takeuchi *et al.,* 2002; Boulbou *et al.,* 2003; Elhaad *et al.,* 2004; Boulbou *et al.,* 2005; Gokulakrishnan *et al.,* 2006).

Data available for patients with GH deficiency that did not receive replacement therapy showed higher E-selcetin concentrations than controls (Gómez *et al.,* 2006). In a previous study after 1 year of GH treatment, no changes in blood pressure were observed and we did not found changes in all these variable including E-selectin whose concentrations were also higher before GH treatment (Gómez *et al.,* 2006). In our series we found no differences in all parameters except in E-selectin concentrations that were higher in Treated group than in Control group in GHD patients and we also found a correlation between E-selectin and ICAM-1 and MCP-1, between IGF-I and the fibrin degradation product D-dimer and an inverse correlation between IGF-I and MCP-1, without correlation between endothelial-mediated vasodilatation response and the fibrinolytic markers.

Recent data in human umbilical vein endothelial cells, endothelial cell activation by endotoxins evidenced by enhanced E-selectin expression is manifested functionally as an increase in monocyte cell adhesion (Alfaro Moreno *et al.,* 2006). On the other hand in baboons fed with a high diet-cholesterol this diet induced a high inflammatory status and membrane-bound E-selectin on endothelial cells were highly increased suggesting a preconditioning atherosclerotic phenomenon (Shi *et al.,* 2005).

Our patients and controls were similar in age, weight, BMI and the other anthropometrical, body composition variables and biochemical characteristics; it is interesting to note this fact because differences in adiposity and obesity may explain some previous findings because obesity is associated with a state of low-grade chronic inflammation as evidenced

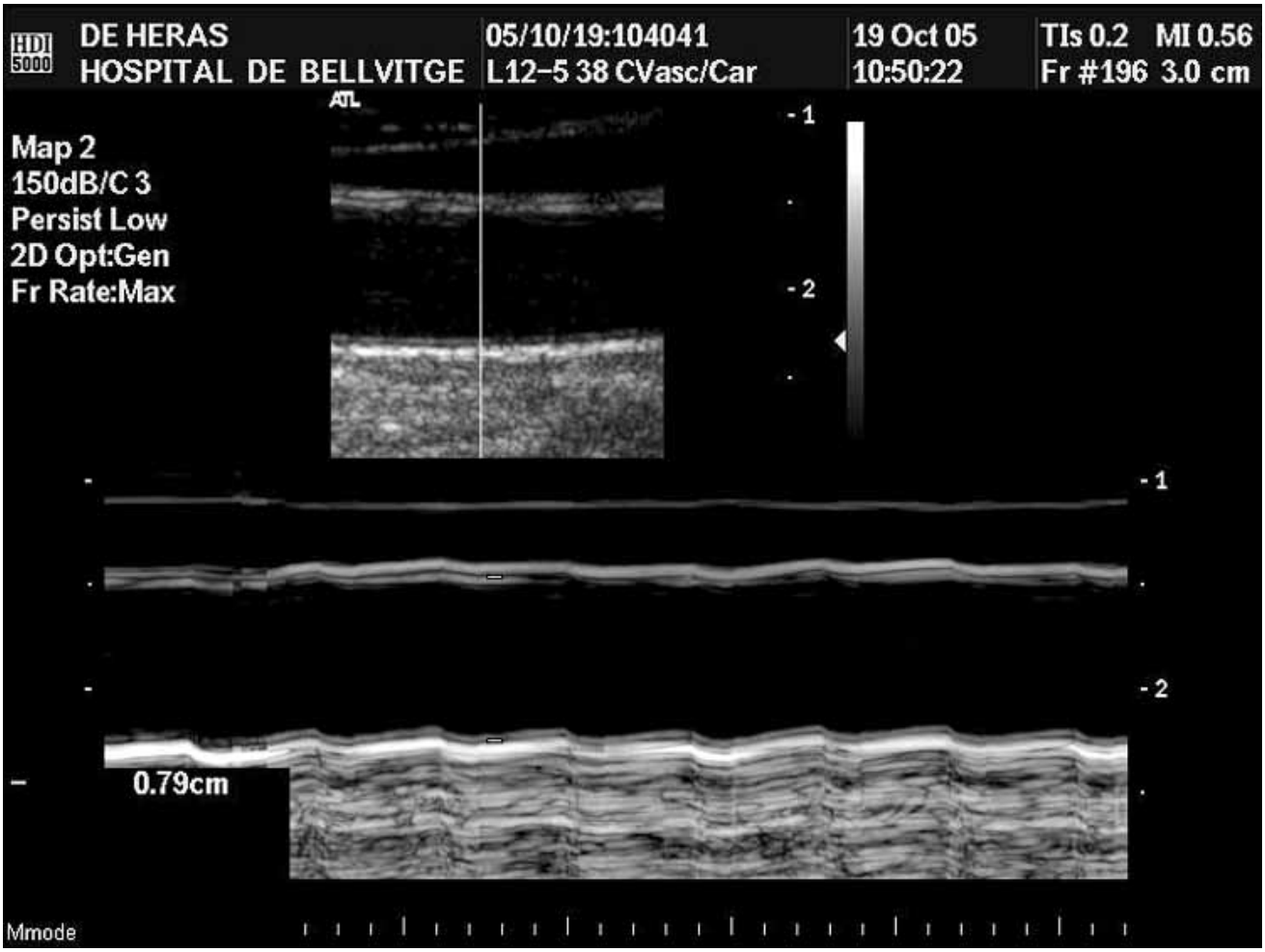

Fig. (1). Doppler tracing in a growth hormone deficiency patients showing humeral artery brachial B-mode image (above) and M-mode (below) with the normal diameter.

by an increase CRP, IL-6 and tumor necrosis factor-α (Ross, 1999; Linton and Fazio, 2003; Bruunsgaar and Pedersen, 2003) and a considerable body of evidence supports the notion that various mediators such as cytokines are involved in the process of atherosclerotic lesions (Ross, 1999; Bruunsgaar and Pedersen, 2003). On the other hand, rising levels of inflammatory markers with ageing is related to the high prevalence of cardiovascular risk factor and mortality (Sesmilo *et al.*, 2000; Ferrucci *et al.*, 2005). All of our patients and controls were non-diabetic, without glucose intolerance or insulinresistance and without family history of diabetes mellitus; it is important in our understanding because previous studies support the idea that even a mild derangement in glucose metabolism may be associated with, or serve as a marker, of detrimental lipid profile, disturbed fibrinolytic markers, soluble adhesion molecules and endothelial dysfunction (Wellen *et al.*, 2005; Gokulakrishnan *et al.*, 2006). Also our GHD patients were no hypertensive; an association of cytokines with systolic and diastolic blood pressure has been previously described (Ross, 1999; Boulbou *et al.*, 2005; Vilarrasa *et al.*, 2006), supporting the hypothesis of a possible role of hypertension as a proinflammatory stimulus. Furthermore, all of our subjects were non-smokers and this is an important fact that can avoid the influence of tobacco consumption over endothelial function (Takeuchi *et al.*, 2002).

In our study, E-selectin concentrations were higher in treated GHD patients than in controls but the present study does not provide direct evidence, only suggestive data, that E-selectin may lead to endothelial injury or dysfunction and that E-selectin is not to be considered a biomarker or indicator of vascular disease from this work and that future studies may support this finding. We can also suggest this fact in GHD patients as consequence of their positive correlation between E-selectin and MCP-1; this chemokine has a potent agonist effect for monocytes, memory T cells and basophils. MCP-1 has been implicated as a key player in the recruitment of monocytes in atheroma, and the over expression of MCP-1 in specific tissues causes a localised infiltration of monocyte and macrophages (Egashira, 2002; Fassahuaer *et al.*, 2004).

In conclusion, in this study we have demonstrated in GHD adults under GH substitution persistent elevated E-

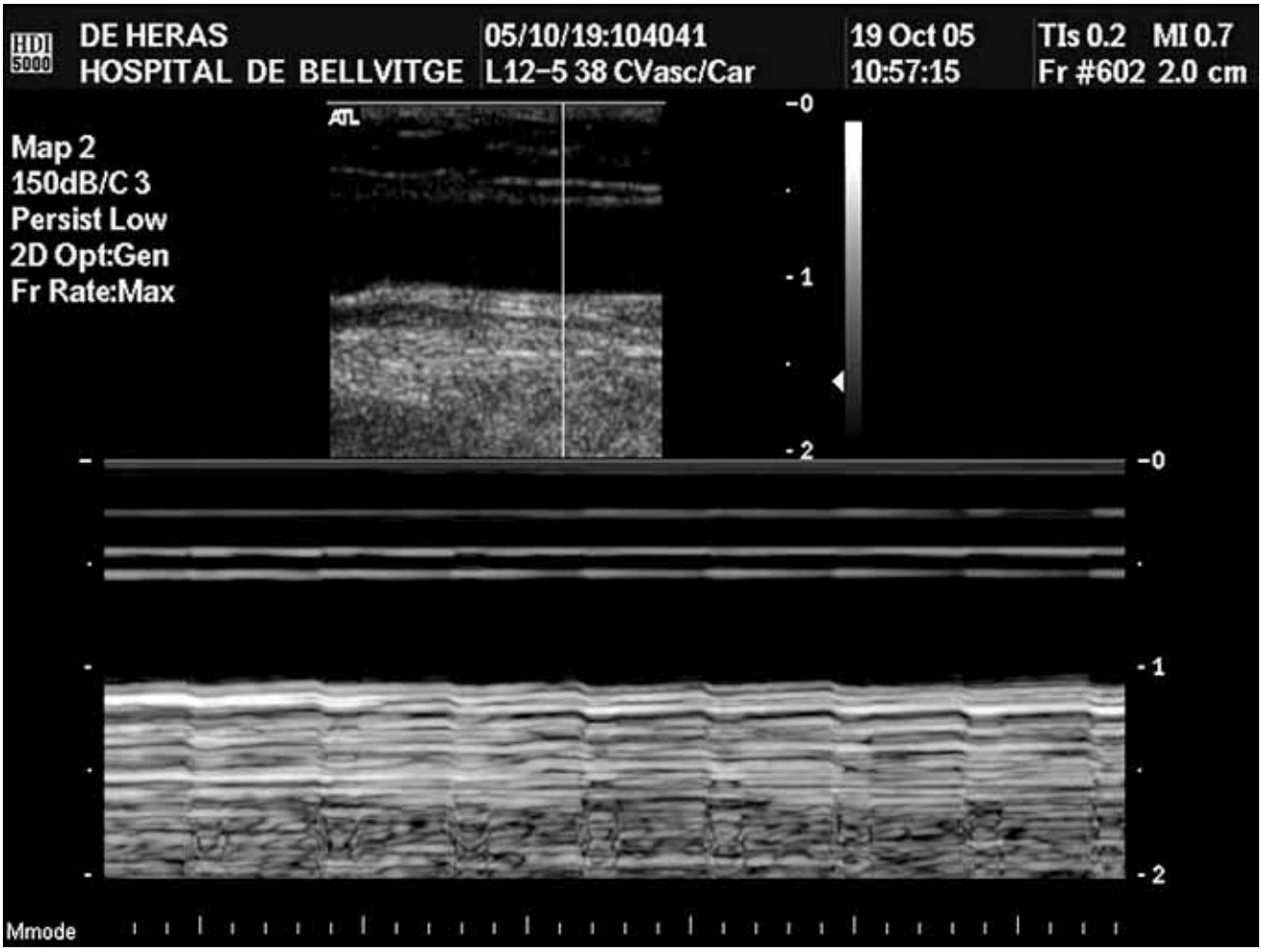

Fig. (2). Doppler tracing in a growth hormone deficiency patients showing humeral artery brachial B-mode image (above) and M-mode (below) with the diameter of the artery 0.79 cm 30 seconds after cuff release and induced hyperemia.

selectin concentrations in a group of patients without differences in age, BMI, and without diabetes mellitus, arterial hypertension or tobacco consumption. This may provide new insights into our understanding of the abnormalities by which vascular events are more frequent in adult patients with hypopituitarism. Furthermore, it will be interesting to consider the improving effect of GH replacement in these patients and that its protective effect may be enhancing other alternative mechanisms.

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