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Serum vascular endothelial growth factor (VEGF) is elevated in GH deficient adults ☆

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ABSTRACT

Objective: GHD adults exhibit a number of adverse surrogate markers of vascular risk culminating in excess vascular morbidity and mortality. Vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) have been implicated in the pathogenesis of a number of vascular morbidities. Furthermore, serum levels decrease following GH replacement in GHD adults, though it remains unclear if levels are significantly elevated in untreated individuals.
Design: A cross-sectional case–control study.
Methods: We measured fasting serum VEGF, MMP2, and MMP9 in 27 patients with GHD, 24 with partial GHD (GHI), and 25 sex- and age-matched controls.
Results: GHD (483 ± 334 vs 326 ± 180 ng/l, P = 0.04), but not GHI (354 ± 192 vs 326 ± 180 ng/l, P = n/s) adults had significantly elevated VEGF levels compared with controls. Neither MMP2, nor MMP9 levels were elevated in the patient groups. Serum VEGF levels correlated positively with LDL-cholesterol (R = 0.34, P = 0.004) and serum MMP9 values (R = 0.36, P = 0.002), and negatively with IGF-I values, however, no correlation was observed with MMP2. Multiple regression analysis with VEGF levels as the dependent variable, and age, gender, % fat mass, LDL-C, insulin and IGF-I as independent variables revealed VEGF levels to be dependent on LDL-C alone (P = 0.003, R = 0.36).
Conclusion: GHD adults have elevated VEGF levels, which correlate with MMP9 levels. Both VEGF and MMP9 are associated with vascular pathologies and may provide insight in to the pathophysiological mechanisms underlying the increased vascular morbidity and mortality observed in GHD adults.

1. Introduction

GHD adults are at increased risk of vascular morbidity and mortality [1–3], and display a clustering of surrogate markers for vascular risk including truncal adiposity [4–6], insulin resistance [7,8], an adverse lipid profile [9,10], and increased pro-coagulant factors [11]. Studies examining the vasculature of GHD adults report endothelial dysfunction [12] and increased arterial intima-medial thickness [13,14]. It is clear, therefore, that the GH axis either directly, or via intermediaries, play an important role in the maintenance of normal vascular functioning.

Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein that plays an important role in angiogenesis, atherogenesis, and vascular remodeling [15]. VEGF induces migration and proliferation of endothelial cells [15–17], migration of monocytes [18,19], increased vascular permeability [15], and increased secretion of matrix metalloproteinases (MMPs) [16]. VEGF has been implicated in the pathophysiology of unstable atherosclerotic plaques [19], and evolution of cerebral infarcts [20]. The MMPs are a family of zinc containing endopeptidases that degrade extracellular matrix (ECM) components. Both MMP2 and 9 degrade gelatins and basement membrane type IV collagen [21]. Elevated serum levels of the gelatinases are observed in patients with angina, acute coronary syndromes, and cardioembolic strokes [22,23]. MMP2 and 9 are also implicated in the pathogenesis of abdominal aortic aneurysms and post-ischaemic myocardial dysfunction [24,25].

GH replacement has been shown to modulate levels of VEGF and MMP2 and 9 [26]. Whether levels of VEGF and MMPs are significantly elevated in untreated severe GHD and partial GHD hypopituitary adults has not been studied. We have, therefore, examined whether

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VEGF, MMP2, and MMP9 levels are abnormal in patients with varying degrees of GHD.

2. Subjects and methods

2.1. Patients

The study cohort comprised three age-, sex-, and BMI-matched groups. Two of the groups were comprised of hypopituitary patients, and the third consisted of healthy subjects drawn from the hospital staff and relatives of participating patients. Consecutive patients attending the Endocrine Investigations Unit at the Christie Hospital for assessment of anterior pituitary function were recruited. Exclusion criteria included renal disease, hepatic disease, diabetes mellitus, ischaemic heart disease, epilepsy, and age >60 years. No patient had received GH replacement therapy in the preceding 12 months. All patients received replacement therapy with hydrocortisone, thyroxine, and sex steroids as required.

Patient groups were defined according to the result of provocative tests of GH secretion. GH status was ascertained in 51 patients at risk of hypopituitarism from a primary hypothalamo-pituitary pathology or cranial irradiation. The GH stimulation test of choice was the insulin tolerance test (ITT, n = 46/51). Where the ITT was contraindicated, and to confirm the patient's GH status, patients underwent alternate stimulation tests using arginine (AST, n = 24), glucagon (GST, n = 9), or GHRH-arginine (n = 16). All patients were required to undergo two tests of GH reserve to confirm their GH status, except in the setting of panhypopituitarism and a peak GH response to the ITT of less than 0.33 μg/l [27].

GHD was defined as a peak GH response of <3 μg/l to the ITT, arginine, and glucagon tests (n = 27) [28]. GH-insufficiency (GHI) was defined by a peak GH response of <7 μg/l to both tests performed, but >3 μg/l to at least one of the two tests (n = 24). Respective values for the diagnosis of GHD and GHI using the GHRH-arginine test were <9, and 9–21 μg/l respectively [29,30].

2.2. Study protocol

Whole body scanning [31] for body composition was assessed using dual energy x-ray absorptiometry as previously described [32]. Carotid artery intima-media thickness (IMT) was measured by high definition ultrasound. The patients attended fasting and blood was drawn. Serum samples were centrifuged and then stored at −80 °C. The study was approved by South Manchester Area Health Authority ethics committee. All subjects were provided with written and verbal information concerning the study, and all subjects gave written informed consent.

2.3. Carotid ultrasonography

Carotid ultrasound was performed using an Acuson XP and a 7.5 MHz Linear array tranducer. The IMT were measured within 2 cm of the carotid bifurcation, taken as the thickest radial dimension of the posterior arterial wall echogenic intima and hypoechoic subintimal layer, recording 3 measurements from each common carotid artery.

2.4. Assays

IGF-I was determined, after acid-alcohol extraction, by IRMA using a commercially available kit (DSL Inc., Webster, Texas). Sensitivity was 0.8 μg/l, and intra-assay coefficients of variation (CVs) at 9.3, 55.3, and 263.6 μg/l were 3.4, 3.0, and 1.5% respectively. Inter-assay CVs at 10.4, 53.8, and 255.9 μg/l were 8.2, 1.5, and 3.7% respectively. Insulin was determined by a commercially available radioimmunoassay (DSL Inc.). Sensitivity was 1.3 mU/l, with intra-assay coefficients of variation (CVs) at 4.8, 17.6, and 54.6 mU/l of 8.3, 4.5, and 6.4% respectively. Inter-assay CVs at 6.1, 41.7, and 101.4 mU/l were 7.7, 9.2, and 5.2% respectively. All samples were assayed in triplicate.

Cholesterol, triglycerides and direct HDL-C assays were carried out on a Bayer ADVIA 1650 chemistry analyzer (Bayer Diagnostics, Newbury, Berks, UK) using proprietary methods. The cholesterol and triglyceride methods, respectively, employ cholesterol oxidase and lipoprotein lipase/glycerol kinase. The HDL-C method measures cholesterol by PEG-linked cholesterol esterase and oxidase after serum incubation with sulphated cyclodextrin buffer. LDL was calculated using the Friedewald equation. LDL was not determined if serum TGs were >4 mmol/l.

Human VEGF (sensitivity <9 ng/L; intra-assay precision (CV), 6.7%) and total MMP-9 (sensitivity 0.156 μg/l; CV, 2.9%) evaluations were performed using commercially available ELISA kits (Quantikine, R & D Systems, USA) following the manufacturer's instructions. Human MMP-2 (sensitivity 0.37 μg/l; CV, 6.3%) measurements were carried out with ELISA kits (Biotrak, Amersham Pharmacia Biotech, UK).

2.5. Statistics

Non-parametric and parametric data are presented as median and inter-quartile ranges, or mean ± standard deviation respectively. Differences between the three groups of data were examined using an ANOVA on ranks or ANOVA respectively. Non-paired data were compared using the Mann–Whitney rank sum test. Correlations were sought using Spearman's test. Forward stepwise multiple linear regression was used to examine determinants of serum VEGF. A P value of <0.05 was deemed significant.

3. Results

3.1. GH status

The study group to which patients were assigned was defined according to the results of GH stimulation tests. Using this classification 27 patients were GHD and 24 GHI. IGF-I levels in the GHD, GHI, and control subjects were 211 ± 116, 295 ± 104, and 367 ± 130 μg/l (ANOVA, P<0.0001) respectively. Demographics of the three groups are summarised in Table 1.

3.2. Body composition, carotid intima-medial thickness (IMT), serum lipids and insulin

Data are presented in full in Tables 1 and 2. There were no differences in age or BMI between the three groups (Table 1). Both

Table 1
Demographic data for the study cohort. The patients were divided according to their GH status.

	n	AO/CO	Age (years)	BMI (kg/m^2)	No. of pituitary hormone deficits
GH Deficient	27 (10 F)	17/10	35.6 ± 11.4	25.7 ± 7.0	GHD0 × 9, GHD1 × 10, GHD2 × 3, GHD3 × 5
GH Insufficient	24 (12 F)	11/13	31.5 ± 13.2	25.0 ± 3.6	GHD0 × 20, GHD1 × 3, GHD3 × 1
Controls	25 [15]	N/A	31.7 ± 12.2	23.8 ± 4.0	N/A

GHD0, GHD1, GHD2, and GHD3 represent 0, 1, 2, and 3 anterior pituitary hormone deficits in addition to GH respectively.
AO, adult-onset; and CO, childhood-onset of pituitary disease.