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Evaluation of coagulation and fibrinolytic parameters in adult onset GH deficiency and the effects of GH replacement therapy: A placebo controlled study

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ABSTRACT

Objective: Increased cardiovascular mortality/morbidity observed in patients with hypopituitarism is ascribed to growth hormone deficiency (GHD) because of its unfavorable cardiovascular risk profile. Abnormalities in the coagulation system may also contribute to increased cardiovascular morbidity/mortality. To get a better insight into the role of hemostasis in GHD we assessed several hemostatic markers at baseline and after 6 months of GH replacement therapy (GHRT).

Design-patients: Nineteen patients with adult onset GHD were enrolled (twelve patients into the treatment and seven patients into the placebo group) into the study. Platelet count, collagen/epinephrine closure time, collagen/ADP closure time, fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin III (AT III), protein C activity, protein S activity, lupus anticoagulant, antiphospholipid antibody immunoglobulin M, and antiphospholipid antibody immunoglobulin G were measured at baseline and 6 months after treatment.

Results: The investigated parameters in the groups were similar at baseline except for low protein S (PS) activity. Protein S deficiency was observed in three of the patients in the GH treatment group at baseline, however the PS activity values normalized following GHRT. AT III and protein C activities decreased when compared to baseline values in the treatment group but not in the placebo group.

Conclusions: We observed protein S deficiency more frequent than seen in the general population and normalization of protein S activity and decreases, in other natural anticoagulants following GHRT. Further studies are required to understand the impact of these changes in cardiovascular morbidity and mortality in this patient population.

1. Introduction

An increased prevalence of cardiovascular diseases in hypopituitary patients has been demonstrated in many studies, and growth hormone (GH) deficiency (GHD) is thought to be responsible for this because of its adverse effects on cardiovascular risk factors such as altered body composition, insulin resistance, dyslipidemia, and hypertension [1–6]. It is well known that ischemic cardiovascular events are mostly triggered by thrombosis after atherosclerotic plaque disruption and thrombi are resolved through the action of the fibrinolytic system [7]. Abnormalities in the coagulation system also seem to contribute to increased morbidity and mortality in cardiovascular diseases [8].

Changes in the coagulation and fibrinolytic systems in growth hormone-deficient patients are of interest because data regarding the exact mechanisms underlying the increased cardiovascular risk observed in these patients are lacking. We previously reported the case of a patient with Sheehan's Syndrome who had massive cardiac thrombosis without any other risk factors for prothrombotic state except GHD [9]. Although there are several studies reporting the effects of various factors that play a role in hemostasis in GH-deficient patients; to our knowledge most of the parameters were separately evaluated in previous studies, we aimed to investigate major determinants of the coagulation/fibrinolytic pathways in the same patient group.

2. Subjects and methods

Nineteen patients with adult onset GHD (7 males and 12 females) with a mean age of 47.7 ± 10.2 years were enrolled into the study (Table 1) between years 2007 and 2009. Platelet count, collagen/epinephrine closure time, collagen/ADP closure time, fibrinogen,

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Table 1
Clinical findings of patients with GHD.

Patient no	Age	Gender	Diagnosis deficient pituitary hormones	Duration
Placebo group				
1	61	F	SS FSH/LH, GH	21 years
2	40	M	PA ACTH, FSH/LH, TSH, GH	5 years
3	36	F	SS FSH/LH, GH	11 years
4	41	M	PA ACTH, FSH/LH, TSH, GH	8 years
5	38	F	SS ACTH, FSH/LH, TSH, GH	10 years
6	41	F	SS ACTH, FSH/LH, TSH, GH	16 years
7	60	M	Trv ACTH, FSH/LH, TSH, GH	25 years
GH receiving group				
8	46	F	SS ACTH, FSH/LH, TSH, GH	20 years
9	45	M	PA ACTH, FSH/LH, TSH, GH	7 years
10	33	F	SS ACTH, FSH/LH, TSH, GH	8 years
11	66	F	SS ACTH, FSH/LH, TSH, GH	26 years
12	37	F	SS ACTH, TSH, GH	5 years
13	57	F	SS ACTH, FSH/LH, TSH, GH	30 years
14	64	F	SS ACTH, FSH/LH, TSH, GH	25 years
15	61	F	SS ACTH, FSH/LH, TSH, GH	20 years
16	37	M	Trv GH	3 years
17	55	F	SS ACTH, FSH/LH, TSH, GH	25 years
18	52	M	LH ACTH, FSH/LH, TSH, GH	3 years
19	40	M	PA ACTH, FSH/LH, TSH, GH	3 years

Duration: Estimated duration of growth hormone deficiency; SS: Sheehans' Syndrome; PA: operated pituitary adenoma; Trv: head trauma; LH: lymphositic hypophysytis; ACTH: Adrenocorticotrophic Hormone; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; TSH: Thyroid Stimulating Hormone; GH: growth hormone.

prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin III (AT III), protein C activity, protein S activity, lupus anticoagulant, antiphospholipid antibody immunoglobulin M, and antiphospholipid antibody immunoglobulin G were evaluated.

Severe growth hormone deficiency was diagnosed in all patients by insulin tolerance tests (ITT) if the peak growth hormone response was less than 9 mIU/l (3 μg/l). Hypopituitarism was due to Sheehan's Syndrome in 12 patients; due to operated pituitary adenoma in 4, due to lymphocytic hypophysitis in 1 and due to head trauma in 2 patients (Table 1). At the time all cases were receiving adequate replacement therapies other than GH for pituitary hormone deficiencies. Four premenopausal female patients were receiving sex steroid (ethinyl estradiol 20 μg and desogestrel 150 μg, PO) replacement. In patients on conventional hormone replacement therapy, basal hormone levels had been stable for at least six months before enrollment (data not shown).

All patients were normotensive, and non-diabetic. Patients, with a history of any disease or receiving drugs known to affect the coagulation/fibrinolytic system were excluded from the study. Twelve patients were randomized into the treatment and seven patients into the placebo group (premenopausal female patients were equally divided between two groups; 2 into the treatment and two into the placebo groups) by computer method. Twelve patients received recombinant GH (Genotropin; Pfizer Stockholm, Sweden) for 6 months (treatment group) and seven patients received a placebo (placebo group) during this period. GH replacement treatment (GHRT) was given according to the recommendations of the Growth Hormone Research Society Workshop [10].

GH was self-administered at night subcutaneously and drug compliance was assessed by vial count. GHRT was started at a dose of 0.45 IU (0.15 mg)/day in the first month, and increased to 0.9 IU (0.30 mg)/day during the second month. The maintenance dose, ranging from 1.5 IU to 1.8 IU/day until the end of sixth month was adjusted according to IGF-I levels for the relevant ages for each patient.

Blood samples were collected by venipuncture at 08.00 h after an 8–10 h overnight fast in tubes containing anticoagulant sodium citrate (volume of citrate/volume of blood:1/9) and centrifuged at 2000×*g* for 15 min. PT (with a Tromborel S kit), aPTT (with a Dade Actin kit), fibrinogen (with a Dade Thrombin kit), protein-S activity (with a Protein S AC® kit), protein-C activity (with a Protein-C Reagent Dade, Behring kit), and antithrombin III (with a Berichrom Antithrombin III kit) were performed using a Sysmex CA.7000 analyzer. Our laboratory references at the time of study were as follows: PT, 10.5–13.2 s; aPTT, 23–35 s; fibrinogen, 146–380 mg/dl; protein C activity, 78–134%; protein S activity, 55–160%; AT III, 63–122%. Platelet functions were detected with PFA-100 using collagen/epinephrine (Col/Epi) and collagen/ADP (Col/ADP) test cartridges with reference ranges of 71–118 and 85–165, respectively. The antiphospholipid antibodies IgM and IgG were detected by ELISA test (Euroimmun). Lupus anticoagulants were measured by a simplified dilute Russell's viper venom test (DRVVT) and accepted as normal within ranges of 0.8–1.2; 1+ within ranges 1.2–1; 2+ within ranges 1.5–2; 2+ above 2.

Serum GH levels were measured by using immunoradiometric assay with a commercial kit (DSL, Webster, TX, USA); intra-assay and inter-assay coefficients of variation were 3.1% and 5.9%, respectively. GH standards were calibrated according to the WHO reference standard 88/624. IGF-I was measured by immunoradiometric assay after formic acid–ethanol extraction (BC1010, Biocode SA, Liege, Belgium); intra-assay and inter-assay coefficients of variation were 3.4% and 8.4%, respectively. The minimum detectable concentration of IGF-I was 5 ng/ml, and the reference ranges (mean − 2 S.D., mean + 2 S.D.) for the relevant ages were 83–570 ng/ml (30–45 years) and 61–430 ng/ml (46–60 years).

The Local Ethics Committee of Erciyes University approved the study. All patients gave their informed consent for participation in the study.

3. Statistical analysis

Statistical analysis was performed using the SPSS 13.0 program. All data were subjected to a Kolmogorov–Smirnov test for normality and presented as mean ± SEM. Because the groups were not normally distributed, Mann–Whitney U and Wilcoxon tests were used to compare the differences between groups and within groups, respectively. $p<0.05$ is accepted as significant.

4. Results

In the treatment group there was a significant increase in the serum IGF-1 level after 6 months of GHRT when compared with the mean baseline IGF-1 level (from 77.5 ± 30.0 to 276.5 ± 33.0 ng/ml, $p=0.02$). No difference was found in the placebo receiving group between the serum IGF-1 levels at baseline and after 6 months (28.7 ± 7.5 and 31.4 ± 10.3 ng/ml, respectively; $p=0.74$) as expected.

4.1. Primary hemostasis

Platelet counts were within normal ranges and there was no difference between the groups at baseline (Table 2). No significant change was observed following GHRT and placebo after 6 months (Tables 3 and 4).

The closure times in response to collagen/ADP were within normal ADP cartridge closure time limits (Table 2) and similar in both groups at baseline. After 6 months no significant change was observed in the GH treatment and placebo groups (Tables 3 and 4). At baseline the mean collagen/epinephrine closure time was higher than the normal reference range in the GH treatment group but there was no difference between the groups (Table 2). After 6 months of GHRT the mean collagen epinephrine closure time was normalized but the decrease did not reach a significant level (Table 3).