

Research paper**Growth hormone binding protein and acid labile subunit levels in the assessment of acromegaly treatment**

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ABSTRACT

OBJECTIVE: Post-treatment monitoring of acromegalic patients is a matter of controversy, as discrepancies between GH and IGF-I levels have been reported. The aim of our study was to evaluate the role of acid-labile subunit (ALS), a component of the 150 kD IGF-I/IGFBP-3/ALS complex, and the growth hormone binding protein (GHBP) in the follow-up of patients with acromegaly after therapeutic intervention. **DESIGN:** Forty-one patients with acromegaly, 10 at the time of diagnosis and 31 post therapeutic intervention, were studied. Patients were evaluated by the determination of baseline (fasting) IGF-I, ALS and GHBP and of glucose and GH during OGTT. **RESULTS:** Significantly lower ALS and higher GHBP levels were detected in successfully treated acromegalics compared to patients before treatment (34.1 ± 1.6 vs. 52.8 ± 2 mg/L and 0.9 ± 0.08 vs 0.4 ± 0.1 μ g/L, respectively $P < 0.05$). Furthermore, no difference was noted in ALS and GHBP values between patients successfully treated with either somatostatin analogues or another type of treatment. **CONCLUSIONS:** a) Successfully treated acromegalic patients demonstrate lower ALS and higher GHBP levels than patients before treatment, and b) somatostatin analogue treatment does not have a direct effect on GHBP and ALS concentration in acromegaly. Studies in larger groups of patients are needed to disclose whether these alterations will be useful in the post-treatment assessment of acromegalic patients.

Key words: Acromegaly, Acid labile subunit, Growth hormone binding protein

INTRODUCTION

Acromegaly is a rare disease which leads to high rates of morbidity and mortality¹. Epidemiological studies have shown that adequate control of acrome-

galy restores mortality rates to those of the general population^{2,3}. GH nadir levels $< 1 \mu$ g/L during an oral glucose tolerance test (OGTT) and normal for age and sex IGF-I levels are the current criteria for acromegaly control^{4,5}. However, it has been demonstrated that 15-30% of acromegalics with normal IGF-I values fail to normalize GH levels and vice versa⁶. Diurnal variation of GH secretion⁷, problems with reproducibility⁸ and sensitivity of GH assays⁹, methodological problems with IGF-I measurements

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due to binding proteins¹⁰ and the direct inhibitory effect of somatostatin and its analogues on IGF-I production¹¹ are factors that account for these discrepancies and create diagnostic uncertainties in the follow-up of acromegalic patients. When GH receptor antagonists are used for the treatment of acromegaly^{12,13}, IGF-I levels are the only tool available to evaluate disease activity; thus, the need to assess the validity of other markers for this purpose is imperative.

Acid-labile subunit (ALS) is a component of the 150 kD IGF-I/IGFBP-3/ALS complex, essential for ensuring constant plasma IGF-I levels¹⁴. It has been shown that ALS is independent of mechanisms that influence IGF-I and IGFBP-3 secretion¹⁵ and is less age-dependent than IGF-I and IGFBP-3¹⁶. Furthermore, unlike IGF-I and IGFBP-3, ALS is not affected by impaired anterior pituitary function¹⁷.

The high affinity Growth Hormone Binding Protein (GHBP) is the soluble extracellular portion of the GH receptor, derived by proteolytic cleavage¹⁸. This protein binds 40-50% of circulating GH and seems to protect GH from elimination and degradation, thus regulating GH action¹⁹. It has been shown that GHBP levels reflect GH tissue receptor status¹⁸. The secretion of GHBP is GH dependent and, as their circulating concentration is relatively stable during the day²⁰, they provide an estimate of the integrated GH secretion.

In acromegaly, decreased GHBP²⁰⁻²² and increased ALS^{16,17,23} levels have been reported. Thus, these two parameters may offer a potentially useful tool in the assessment of disease activity in acromegaly after therapeutic intervention.

The aim of our study was to evaluate the effect of acromegaly treatment on ALS and GHBP levels and their differential role in assessing treatment efficacy of patients with acromegaly. We also examined the differential effect of somatostatin analogues and other treatment modalities on ALS or GHBP levels in acromegalic patients.

PATIENTS AND METHODS

A total of 41 patients with acromegaly were included in the present retrospective, cross-sectional

study. The diagnosis of acromegaly was made on the basis of signs and symptoms of the disease, elevated random GH levels ($>2.5 \mu\text{g/L}$), not adequately suppressed (below $1 \mu\text{g/L}$) by an oral glucose load, (OGTT) elevated IGF-I levels and radiological detection of a pituitary adenoma⁴.

The study group included 10 patients evaluated at the time of diagnosis and before any therapeutic intervention (pretreatment group), and 31 patients evaluated after therapeutic intervention. Fourteen out of the 31 patients had normal IGF-I values for age and sex and GH levels $<1 \mu\text{g/L}$ during OGTT post treatment, and were considered in remission (successfully treated group), whereas 17 patients failed to meet the above criteria^{4,5} and were designated as an unsuccessfully treated group. Of the 23 acromegalics who achieved normal IGF-I for age and sex post treatment, 9 patients failed to meet all the current consensus criteria for acromegaly control, as they demonstrated inadequate GH suppression (GH $>1 \mu\text{g/L}$) during an OGTT. Details on anthropometric, biochemical and hormonal characteristics of the 3 groups of patients are depicted in **Table 1**. The study was approved by the local ethical committee and consent was obtained from the patients involved.

The 31 patients studied post-treatment had been submitted to different therapeutics modalities (transsphenoidal surgery, radiotherapy and somatostatin analogue administration) according to standard treatment algorithms¹² and were evaluated at least 3 months after surgery or initiation of somatostatin analogue treatment and 3 years after radiation treatment. Slow-release long-acting somatostatin analogues were used (Sandostatin LAR 20 or 30 mg im every 4 weeks, Novartis, and Somatulim 30mg every 2 weeks, Ipsen, France).

All patients were ambulatory. Patients with pituitary hormones deficiencies were on standard replacement treatment. GH levels were measured under baseline conditions and the mean GH value of at least two samples were recorded. OGTT was performed as follows: After an overnight fast, a blood sample was drawn between 08:30 and 09:00 for baseline (fasting) determination of glucose, GH, IGF-I, ALS and GHBP and at 30, 60, 90 and 120

Table 1. Clinical characteristics and hormonal data of the 3 groups of patients with acromegaly

	Before treatment	Successfully treated	Unsuccessfully treated
n	10	14	17
Age (years)	52.9±4	48.1±3.8	51.9±2.8
M/F	2/8	5/9	8/9
Micro/macro	4/6	5/9	7/10
BMI	27.4±1	26.4±0.8	27.8±0.85
W/H	0.86±0.03	0.83±0.02	0.87±0.02
Mean GH (µg/L)	32.4±14.6	1.04±0.2 ^a	8.86±3.59 ^b
GHn (µg/L)	21.8±12.7	0.5±0.06 ^a	4.9±2.2 ^b
IGF-I (µg/L)	545±58.9	179.9±16.3 ^a	321.7±42.4 ^b
Treatment			
TSS	-	5	-
TSS+XR	-	4	2
TSS+SMS	-	2	9
SMS	-	3	6

M/F= male to female ratio, micro/macro= microadenomas to macroadenomas ratio, BMI= body mass index (weight (kg)/height (m)²), GHn= GH nadir during OGTT, TSS= transsphenoidal surgery, XR= radiotherapy, SMS= somatostatin analogue,

W/H=waist to hip ratio

a: statistically significant difference compared to values of patients before treatment

b: statistically significant difference compared to values of successfully treated patients

min after glucose load (75 g of dextrose) for the determination of glucose and GH.

Hormone assays

Samples were collected and centrifuged and serum was stored in aliquots at -20 C° until assayed. All samples were processed in duplicate.

Serum GH concentrations were determined by a commercially available immunoradiometric assay (IRMA) kit (CIS bio international, Gif-sur-Yvette, France). The minimum detection limit was 0.04 µg/L. The intra-assay coefficient of variation was 2.4% and 2.8% at a concentration of 3.46 µg/L and 17 µg/L, respectively. Serum IGF-I was measured by a commercially available immunoradiometric assay (IRMA) kit (Nichols Institute, San Juan Capistrano, CA, USA). This assay uses large excess of IGF-II to block IGF-BPs and has been validated against

the Nichols acid-ethanol extraction RIA. The intra-assay coefficient of variation was 4.6%, 3.3% and 4.1% at a concentration of 60.1 µg/L, 312 µg/L and 594.3 µg/L, respectively.

GHBP levels were determined by enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic System Laboratories, Inc., Webster, TX, USA). The intra-assay coefficient of variation was 5.4%, 1.8% and 5.1% at a concentration of 0.82 µg/L, 3.83 µg/L and 10.4 µg/L, respectively.

ALS levels were determined by a specific two-site sandwich enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic System Laboratories, Inc Webster, TX, USA). All samples were pretreated in order to dissociate the complex and enhance ALS immunoreactivity. The detection limit was 0.07 mg/L. The intra-assay coefficient of variation was 6.1% and 3.8% at a concentration of 1.65 mg/L and 29.17 mg/L, respectively.

Statistical analysis

Data analyses were performed with a SAS statistical program. Statistical analysis to identify differences between groups of patients was performed with one way ANOVA followed by Student-Newman-Keuls test for comparisons between means or one way ANOVA on the ranks followed by Dunn's test for comparisons between means when data distribution was not normal. Spearman's correlation coefficient was used to evaluate the relationship between values. Data are expressed as mean ±SE. The level of significance was set at 0.05 for all statistical tests.

RESULTS

ALS and GHBP levels

Individual ALS levels of all patients in the 3 groups are shown in **Figure 1**. Mean ALS levels were significantly (P <0.05) lower in the 14 successfully treated acromegalic patients, (34.1±1.6 mg/L) compared to the 10 patients of the pretreatment group (52.8±2 mg/L). Also, significantly (P <0.05) lower ALS levels were noted in the 14 successfully treated patients (34.1±1.6 mg/L) compared to the 17 unsuccessfully treated patients (42.4±2.2 mg/L).

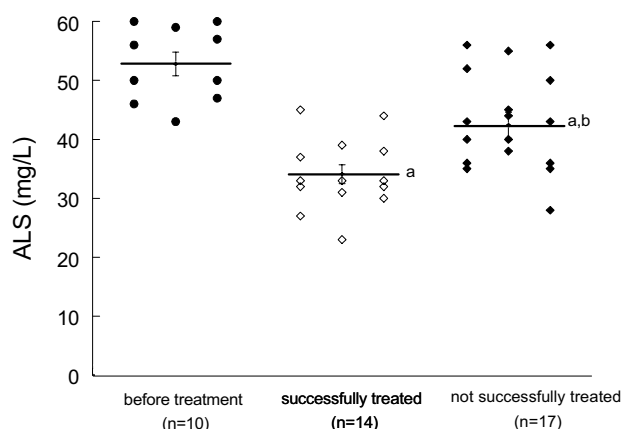


Figure 1. Individual Acid-labile subunit (ALS) levels in patients with acromegaly before treatment (●, n=10), and in successfully (◇, n=14) or not successfully treated (◆, n=17) patients. Horizontal bars indicate mean value \pm SE for the respective group.

a: denotes statistically significant difference compared to patients before treatment ($P < 0.05$)

b: denotes statistical significant difference compared to successfully treated patients, ($P < 0.05$).

As shown in **Figure 2**, mean GHBP levels were significantly ($P < 0.05$) higher in successfully treated acromegalics ($0.9 \pm 0.08 \mu\text{g/L}$), compared to patients before treatment ($0.4 \pm 0.1 \mu\text{g/L}$), and to unsuccessfully treated patients ($0.6 \pm 0.1 \mu\text{g/L}$, $P < 0.05$). No difference in GHBP levels was noted between patients before treatment and unsuccessfully treated acromegalic patients.

In acromegalic patients post treatment, a statistically significant positive correlation was disclosed between ALS, GH ($R=0.52$, $P=0.002$), IGF-I ($R=0.68$, $P=0.0001$) and GH nadir values during an OGTT ($R=0.48$, $P=0.02$), while a significant negative correlation was disclosed between GHBP, GH ($R=-0.38$, $P=0.04$), and IGF-I levels ($R=-0.62$, $P=0.0001$). No correlation was found between either ALS or GHBP and age, body mass index (BMI) or Waist to Hip ratio.

Somatostatin effect on ALS and GHBP levels

To examine whether the alterations of ALS and GHBP levels after therapeutic interventions as described above were influenced by somatostatin administration, successfully treated acromegalic patients on slow-released long-acting somatostatin

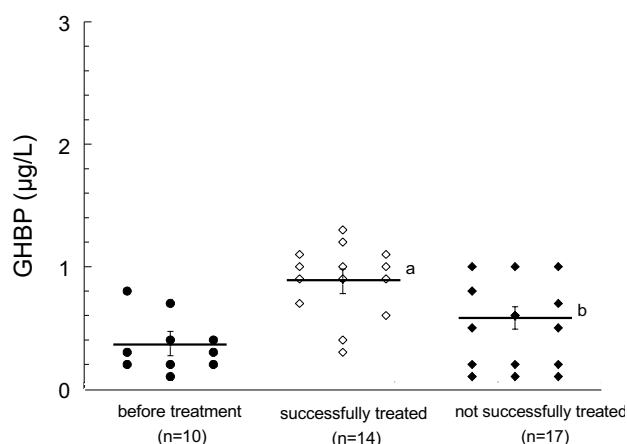


Figure 2. Individual growth hormone binding protein (GHBP) levels in patients with acromegaly before treatment (●, n=10), and in successfully (◇, n=14) or not successfully treated (◆, n=17) patients. Horizontal bars indicate mean value \pm SE for the respective group.

a: denotes statistically significant difference compared to patients before treatment ($P < 0.05$)

b: denotes statistically significant difference compared to successfully treated patients, ($P < 0.05$).

analogues (n=5) were compared with acromegalics successfully treated by transphenoidal surgery or pituitary irradiation (n=9). No differences in ALS or GHBP levels were disclosed between these 2 groups (**Table 2**). As expected, statistically significant differences ($P < 0.05$) in ALS and GHBP values were found between patients successfully and unsuccessfully treated with somatostatin analogues (31.6 ± 1.3 vs $43.2 \pm 2.3 \text{ mg/L}$ for ALS, and 1.08 ± 0.04 vs $0.5 \pm 0.11 \mu\text{g/L}$ for GHBP, **Table 2**).

DISCUSSION

In most studies on mortality and morbidity related to acromegaly, random GH and IGF-I levels^{2,3} have been used as indices of treatment efficacy. Recent studies, however, provide evidence for the necessity of using GH nadir values during an OGTT in the long-term follow-up of acromegalic patients post therapy. Thus, nadir GH levels $< 0.3 \mu\text{g/L}$ are considered a good predictor of long-term remission of the disease^{9,24,25}. The baseline IGF-I and GH values during OGTT are the two parameters used in the assessment of disease activity in acromegaly after treatment with either transphenoidal surgery,

Table 2. Clinical characteristics and hormonal data of patients with acromegaly after treatment

	Unsuccessfully treated (+SMS)	Successfully treated (+SMS)	Successfully treated (-SMS)
n	15	5	9
Age (years)	51.3±2.9	53.7±7.4	45.6±4.5
Mean GH (µg/L)	9.8±4	1.1±0.2 ^a	0.9±0.3 ^a
GHn (µg/L)	5.4±2.5	0.64±0.1 ^a	0.4±0.05 ^a
IGF-I (µg/L)	340.6±45.8	174.4±21.3 ^a	183±23.4 ^a
ALS (mg/L)	43.2±2.3	31.6±1.3 ^a	35.5±2.3 ^a
GHBP(µg/L)	0.5±0.11	1.08±0.04 ^a	0.9±0.1 ^a

+SMS acromegalic patients on slow-release long-acting somatostatin analogues treatment

-SMS = acromegalic patients who never received slow-release long-acting somatostatin analogues treatment, GHn = GH nadir during OGTT

a: statistically significant difference compared to values of patients unsuccessfully treated with SMS, P <0.05

radiotherapy or somatostatin analogue administration²⁶⁻²⁸. As plasma IGF-I levels and GH suppressibility to glucose represent different facets of the disease (i.e. overall GH hypersecretion and GH secretory dysregulation, respectively)²⁹, discrepancies between GH and IGF-I measurements are not uncommon⁶, creating diagnostic problems in the follow-up of acromegalic patients. In the present study we analysed data derived from acromegalic patients irrespective of treatment modality, as the above-mentioned criteria for post treatment disease activity assessment refer to acromegalics submitted to any kind of treatment (surgery, radiotherapy and/or somatostatin analogue). We found that 9 patients, who failed to suppress GH levels, exhibited normal IGF-I levels, indicating low specificity of IGF-I to accurately identify disease persistence according to the currently accepted criteria^{4,5}. Such findings will lead to diagnostic uncertainties, especially in cases in which GH measurements cannot be used (i.e. in patients on GH receptor antagonist treatment) and evaluation of the effect of treatment is based only on IGF-I levels. Because of these uncertainties, we examined the potential use of ALS and GHBP as markers of disease activity in acromegaly after therapeutic intervention.

In agreement with other studies^{16,17,20-23}, we found that successful treatment of acromegaly either by surgery, radiotherapy or somatostatin analogues leads to lower ALS and higher GHBP levels compared to patients before treatment. The use of ALS or GHBP in the assessment of acromegaly activity post treatment has been evaluated against the current criteria for disease control^{7,8} in only two reports^{23,30}. Hernandez et al. demonstrated that after treatment, GHBP levels became normal, particularly in those patients with undetectable GH levels during OGTT³⁰. More recently, it was shown that pathologically elevated IGF-I and/or ALS values indicated increased disease activity, irrespective of GH values²³. It must be underlined that overlap of both ALS and GHBP levels between successfully and unsuccessfully treated acromegalics was disclosed in our study and therefore these two parameters cannot be used independently to assess activity of acromegaly.

In the present study we also addressed the question of a direct effect of somatostatin analogues on ALS and GHBP synthesis and release, independent of GH levels. Such a direct inhibitory effect has recently been demonstrated on IGF-I production from hepatocytes¹¹, as well as on other members of the IGF-I family such as IGFBP-1, both *in vitro*³¹ and *in vivo*³². Furthermore, a dose-dependent inhibitory effect of somatostatin on ALS secretion was found in hepatocyte culture¹⁵. In the present study we found that controlled acromegalics exhibited statistically similar alterations of ALS and GHBP, independently of treatment modality (transsphenoidal surgery or radiation or somatostatin analogue administration), providing evidence against an *in vivo* direct inhibitory effect of somatostatin upon ALS and GHBP secretion. However, the number of our patients being successfully treated with somatostatin analogues is too small to allow for definitive conclusions.

The results of the present study indicate that successful treatment of acromegaly reverses the GHBP and ALS alterations caused by the disease. Furthermore, treatment with slow-release long-lasting somatostatin analogues does not seem to have a direct effect on GHBP and ALS concentration in these

patients. Larger studies will most likely provide stronger evidence as to whether these parameters are helpful in the post-treatment assessment of acromegaly.

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