Comparative Study of Insulin-Like Growth Factor-I (IGF-I) and IGF-Binding Protein-3 (IGFBP-3) Level and IGF-I/IGFBP-3 Ratio Measurements and Their Relationship With an Index of Clinical Activity in the Management of Patients With Acromegaly

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To evaluate the utility of measuring the serum insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) level and the IGF-I/IGFBP-3 ratio in the management of acromegalic patients, we comparatively studied the basal concentration of the aforementioned parameters with determination of plasma IGF-I levels and an index of clinical activity of acromegaly in 16 newly diagnosed acromegalic patients (aged 34 to 64 years) before and after hypophysectomy. After adenomectomy, 10 patients remained with "active" disease and six were "cured." Twenty-nine healthy sex- and age-matched volunteers were also studied. Comparison of individual values between untreated acromegalic patients and control subjects showed that none of the patients had overlapping values for IGF-I, whereas five of 16 and three of 16 patients had overlapping values for serum IGFBP-3 and IGF-I/IGFBP-3 ratio, respectively. When we compared the study parameters between the patients who remained with active disease after adenomectomy and the controls, two of 10 had overlapping values for IGF-I, but six of 10 and five of 10 had overlapping values for serum IGFBP-3 and IGF-I/IGFBP-3 ratio, respectively. Moreover, comparison of these parameters between cured and active patients after hypophysectomy showed that none had overlapping values for IGF-I, whereas three of six and one of six had overlapping values for serum IGFBP-3 and IGF-I/IGFBP-3 ratio, respectively. All biochemical variables studied showed significant relationships with an index of clinical activity of disease. In conclusion, our results seem to indicate that among determinations of plasma IGF-I, serum IGFBP-3, and the ratio IGF-I/IGFBP-3 in the evaluation of acromegalic patients, measurement of the plasma IGF-I level has the most discriminative value in the management of these patients.

DIAGNOSIS OF ACROMEGALY is based on a typical clinical presentation associated with a failure to suppress growth hormone (GH) levels to 2 μg/L or less after a glucose load.[1] However, GH concentration determined by conventional methods is not absolutely reliable, since GH activity is pulsatile.[2] Frequent blood sampling to measure GH levels has been demonstrated to be a convenient way to obtain information about both the total amount of GH and the pattern of GH secretion, but is not practical in most centers.[3]

The concentration of insulin-like growth factor-I (IGF-I) is an integrated measure of GH activity that correlates with 24-hour GH secretion and has been used effectively to diagnose acromegaly.[4,5] IGF-binding protein-3 (IGFBP-3) is the major circulating form of IGFBPs. Since it is GH-dependent but has a prolonged half-life compared with GH and does not exhibit pulsatility, it is also an integrated marker of somatotrope function. Unlike IGF-I, IGFBP-3 circulates in high concentrations and can be reliably assayed from a small sample volume. Determination of IGFBP-3 has been used in the diagnosis of GH deficiency in children,[6,7] and it has also been shown that acromegalic patients exhibit elevated levels.[8] Therefore, determination of IGFBP-3 levels might also be useful in the clinical assessment of GH excess.

IGFBP-3 binds both IGF-I and IGF-II. Previous studies have reported normal levels of IGF-II in acromegaly[9] and an inverse relationship between serum GH levels and low–molecular-weight IGFBPs (IGFBP-1 and -2).[10] Therefore, it has been speculated that the ratio between IGF-I and IGFBP-3 could reflect free, biologically active IGF-I. Its use might provide additional information in the biochemical assessment of acromegalic patients.[11]

The aim of the present investigation was to evaluate the utility of serum IGFBP-3 and IGF-I/IGFBP-3 ratio determinations in comparison to measurement of IGF-I levels and to an index of clinical activity in the management of acromegalic patients.

SUBJECTS AND METHODS

Sixteen previously untreated patients (five men and 11 women) with newly diagnosed active acromegaly were studied. They were aged 34 to 64 years (mean ± SD, 47.1 ± 7.6). The diagnosis of acromegaly was based on typical clinical manifestations, elevated IGF-I levels, and nonsuppressible GH following oral glucose loading. A pituitary tumor was demonstrated in all patients by computed axial tomography or magnetic resonance imaging of the sellar area. Patients were well-nourished and euthyroid, and none had renal failure.

The patients were surgically treated by the transsphenoidal route. All had somatotrope adenoma demonstrated by pathologic study. After adenomectomy, five patients (one from the "cured" and four from the "active" groups) received substitutive hormone therapy with thyroxine, hydrocortisone, and sex steroids. Patients did not take any medication that could have modified the hormonal determinations.

All subjects provided informed written consent before participation in the study protocol, which was approved by the Ethics Committee of General Hospital of Vigo.

Comparisons of plasma IGF-I, serum IGFBP-3 levels, and the IGF-I/IGFBP-3 ratio among the following groups of subjects were made: 29 healthy volunteers (13 women and 16 men) aged 24 to 70 years (41.9 ± 13.5) as controls (group A); six patients who were cured after hypophysectomy (group B); 10 patients who remained with "active" acromegaly after hypophysectomy (group C); and 16 un-

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Table 1. GH, IGF-I, and IGFBP-3 Levels and IGF-I/IGFBP-3 Ratio in the Study Groups

Group	Age (yr)	Sex (M/F)	Index of Clinical Activity	GH (ng/mL)	IGF-I (μg/L)	IGFBP-3 (mg/L)	IGF-I/IGFBP3 Ratio (μg/mg)
A (n = 29)	41.9 ± 13.5 (24-70)	13/16	0	1.00 ± 0.65 (0.25-2.2)	235.8 ± 89.6 (100-434.5)	4.16 ± 0.67 (2.93-5.74)	57.27 ± 21.53 (22.16-90.16)
B (n = 6)	51.5 ± 13.9 (33-75)	1/5	0.33 ± 0.52 (0-1)	1.18 ± 1.10 (0.25-3.1)	241.1 ± 103.5 (77.6-398.0)	4.34 ± 1.04 (2.28-5.05)	53.76 ± 17.63 (34.04-86.71)
C (n = 10)	48.2 ± 9.5 (34-63)	4/6	2.20 ± 0.63 (1-3)	6.48 ± 6.71 (1.4-22.4)	649.7 ± 234.6*† (400-1,032)	6.28 ± 2.01* (4.5-11.0)	105.40 ± 33.68*† (73.43-180.56)
D (n = 16)	47.1 ± 7.6 (34-64)	5/11	2.56 ± 0.51 (2-3)	32.23 ± 42.80 (5.14-177)	940.6 ± 159.1*† (637-1,225)	7.78 ± 1.98* (5.16-11.6)	120.62 ± 28.62* (87.81-182.56)

NOTE. Values are the mean ± SD (range).

*$P < .01$ *v* control group.

†$P < .01$ *v* previous group.

treated acromegalic patients (group D). Groups B and C were evaluated 3 months after hypophysectomy.

The criteria for cure after hypophysectomy was a GH level less than 2 μg/L after oral glucose loading. Patients were tested after an overnight fast at 8:30 AM, with a single blood sample taken to determine IGF-I and IGFBP-3.

The index of clinical activity of acromegaly was based on Lucas Morante's study.[12] Three groups of symptoms or signs that had different scores were considered. Group I comprised acral enlargement, excessive sweating, and soft-tissue swelling, each given a score of 0 to 3. The maximal score for this group was 9. Group II, tiredness and carpal tunnel syndrome, was scored as 0 to 1.5. The maximal score for this group of manifestations was 3. Group III, headache, hypertension, visceromegaly, impaired glucose tolerance, arthropathy, acroparesthesiae, and hirsutism, was evaluated and scored as 0 to 0.5 for each parameter. The maximal score for this group was 3.5.

According to this index, acromegalic patients were classified as follows: (0) inactive, score 0 to 3.5; (1) mild, score 4 to 5.5; (2) moderate, score 6 to 7.5; and (3) severe, score more than 8. The index of clinical activity was determined by the same physician (C.P.) for all study subjects.

Assays

Serum GH level was measured by a highly sensitive immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). Plasma IGF-I was determined by radioimmunoassay (RIA) (Nichols Institute) after acid-ethanol extraction. The sensitivity of the assay was 0.06 μg/L, with an interassay coefficient of variation (CV) of 5.2% to 6.8% for a range of 121 to 641 μg/L and an intraassay CV of 2.4% to 3.8% for a range of 110 to 562 μg/L. Serum IGFBP-3 was determined by RIA (Mediagnost, Tübingen, Germany). The sensitivity of the assay was 0.06 μg/L and half-maximum displacement occurred at 6 μg/L, with an interassay CV of 4.5% to 5.9% for a range of 2.6 to 4.5 mg/L and an intraassay CV of 2.8% to 3% for a range of 2.2 to 4.3 mg/L.

Data Analysis

The results are expressed as the mean ± SD. Comparison of mean values was performed with the Mann-Whitney test. The relationship between biochemical parameters and the index of clinical activity was assessed by ANOVA. Statistical significance was defined at P less than .05.

RESULTS

Comparison of Mean Values

The mean value for plasma IGF-I in the untreated group was significantly higher than in the group considered active after hypophysectomy ($P < .001$); this group had higher mean values than both the cured and control groups ($P < .0001$; Table 1).

With regard to serum IGFBP-3 mean levels, significant differences were observed between either the untreated or active groups versus the control group, but not between either the untreated group versus the active group or between the active group versus the cured group (Table 1). As for the IGF-I/IGFBP-3 ratio, the untreated and active groups had a higher mean value than the cured and control groups, but differences between the untreated group and the active group were not seen (Table 1).

Comparison of Individual Values

None of 16 untreated patients had overlapping values for plasma IGF-I with respect to the control subjects, whereas five of these 16 patients with untreated acromegaly had overlapping values for serum IGFBP-3 with respect to the control subjects. Similarly, three of these 16 untreated patients had overlapping values for the IGF-I/IGFBP-3 ratio with respect to the control subjects (Fig 1).

Since IGF-I and IGFBP-3 values decrease with age, we also compared the individual values for both parameters between acromegalic and control subjects divided by age up to 55 years and age above 55 years. In 14 untreated acromegalics aged 34 to 54 years, IGF-I levels ranged from 637 to 1,225 μg/L and did not overlap with the range in the control group until 55 years, 115 to 434.5 μg/L. Two patients aged 56 and 64 years had IGF-I levels of 1,102 and 558 μg/L, respectively, and again these did not overlap with the range for control subjects older than 55 years, 100 to 263 μg/L.

With regard to IGFBP-3 levels, in 14 untreated acromegalics aged 34 to 54 years, IGFBP-3 values ranged from 5.16 to 11.6 mg/L, and four of 14 had overlapping values with the range for control subjects up to 55 years, 2.93 to 5.74 mg/L. The two acromegalics older than 55 years had IGFBP-3 levels of 11.2 and 5.12 mg/L, one of which overlapped with the range for control subjects older than 55 years, 2.36 to 5.14 mg/L.

When individual values for patients with active disease after hypophysectomy were compared with the values for normal subjects, two of 10 patients had overlapping values for IGF-I, six of 10 for IGFBP-3, and five of 10 for the IGF-I/IGFBP-3 ratio (Fig 1).

Moreover, a comparison of individual values between patients with active acromegaly after hypophysectomy and cured

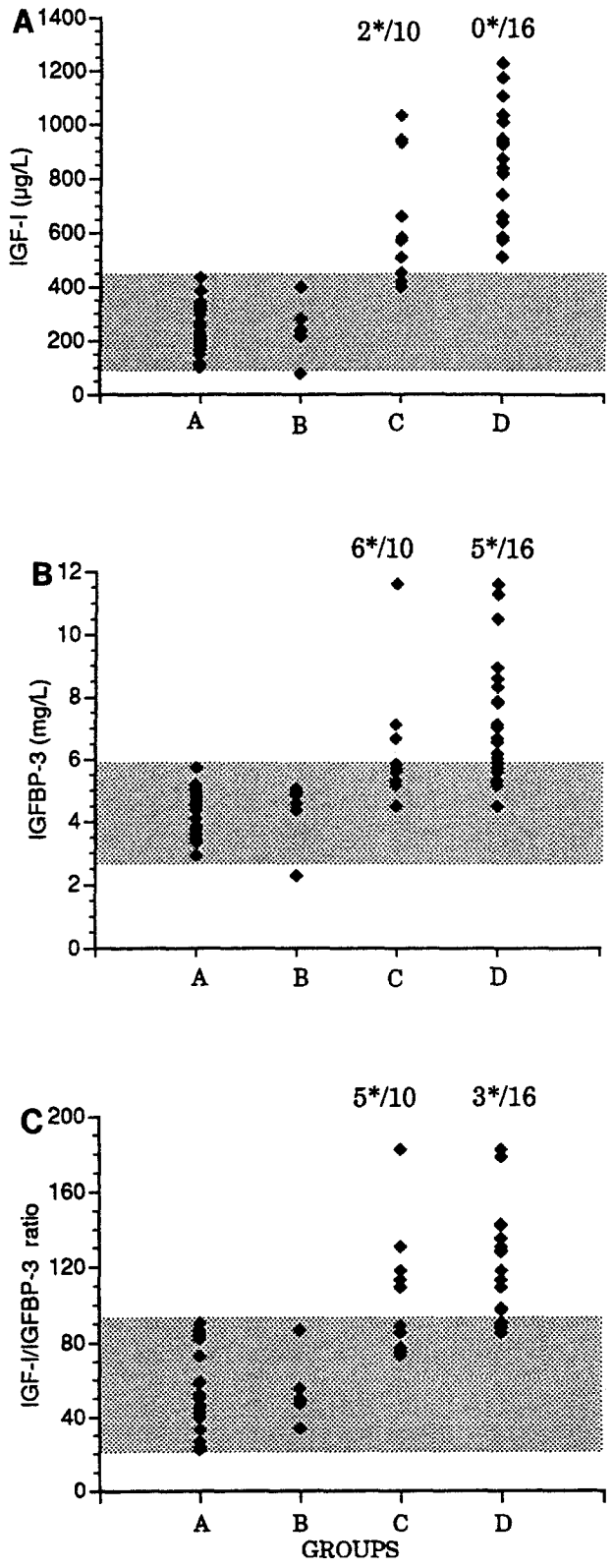

Fig 1. Comparative study of individual values for IGF-I (A), IGFBP-3 (B), and IGF-I/IGFBP-3 ratio (C) between study groups A, B, C, and D. *Overlap with control levels.

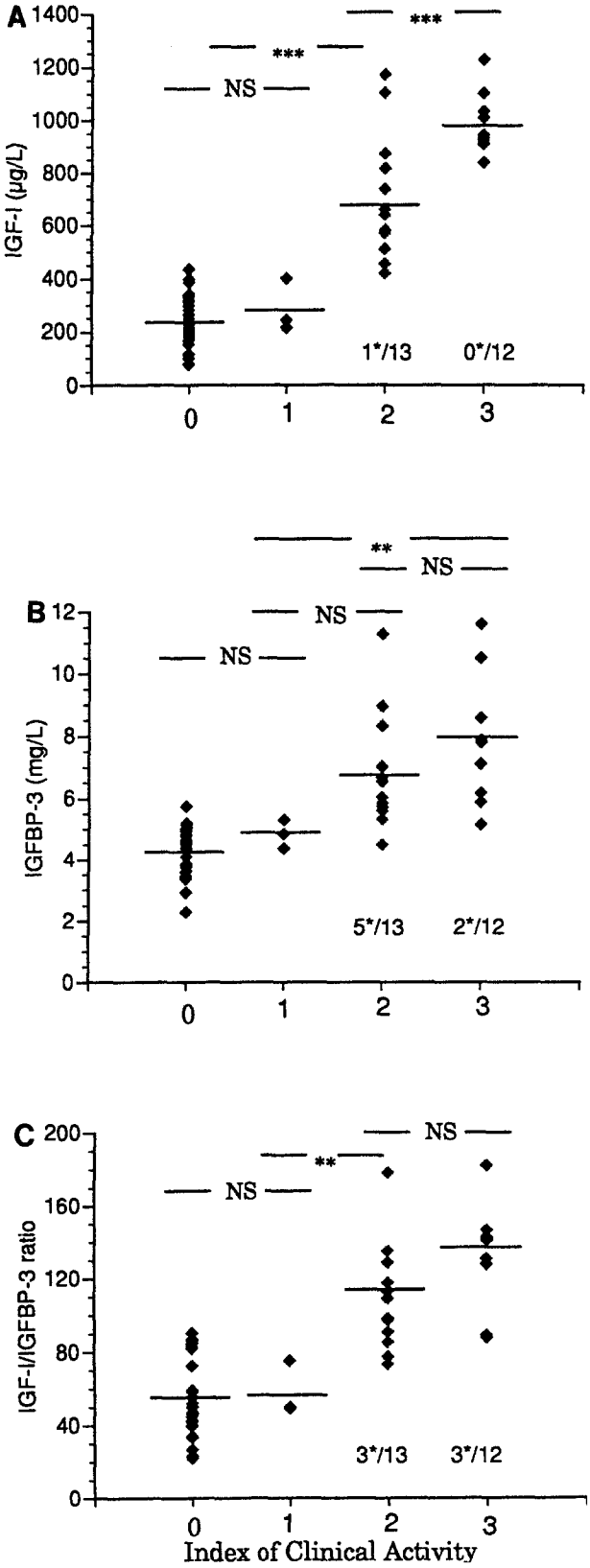

Fig 2. Comparative study of individual values for IGF-I (A), IGFBP-3 (B), and IGF-I/IGFBP-3 (C) ratio in study subjects grouped according to an index of clinical activity of acromegaly (inactive = 0, mild = 1, moderate = 2, severe = 3). *Overlap with subjects without active disease. $^{**}P < .001$, $^{***}P < .0001$.

patients showed that none of these patients had overlapping values for IGF-I with the active subjects, whereas three of six and one of six had overlapping values for serum IGFBP-3 and the IGF-I/IGFBP-3 ratio, respectively (Fig 1).

Relationship Between the Biochemical Parameters and an Index of Clinical Activity of Acromegaly

We observed a significant relationship between an index of clinical activity of the disease and plasma IGF-I ($F = 104,3$, $P < .0001$), serum IGFBP-3 ($F = 24.17$, $P < .0001$), and the IGF-I/IGFBP-3 ratio ($F = 32.7$, $P < .0001$). Subsequently, we grouped the study subjects according to an index score of clinical activity of acromegaly. Comparison of individual values for plasma IGF-I, serum IGFBP-3, and the IGF-I/IGFBP-3 ratio between patients with moderate [2] or severe [3] clinical activity and the group of subjects without acromegalic activity also showed that IGF-I had fewer overlapping values than IGFBP-3 and the IGF-I/IGFBP-3 ratio (Fig 2).

DISCUSSION

The results of the present study indicate that determination of plasma IGF-I has more discriminative value than determination of serum IGFBP-3 for the evaluation of acromegalic patients, since there were no overlapping values for plasma IGF-I between 16 untreated acromegalic patients and age-comparable controls, whereas determination of serum IGFBP-3 showed some overlapping values between untreated patients and normal controls. This difference remained when we compared the individual values for both biochemical parameters between acromegalics and controls grouped for age. This conflicted with a recent report by Grinspoon et al,[13] which observed no overlapping values for serum IGFBP-3 between patients with untreated acromegaly and control subjects. Differences in assay methodology may help to explain the conflicting results between our study and that of Grispoon et al, since our study had a broader range of normal values for serum IGFBP-3. However, in line with our observation, de Herder et al[14] observed a considerable overlapping of values for serum IGFBP-3 between patients with untreated acromegaly and controls.

Furthermore, our investigation extends the aforementioned data to postsurgical evaluation of acromegalic patients. We observed fewer overlapping values for plasma IGF-I versus serum IBFBP-3 in patients who remained with active disease after hypophysectomy compared with the control subjects. The same was seen on comparison of individual values between cured and active patients after hypophysectomy. Moreover, comparison of individual values for IGF-I and IGFBP-3 in the study subjects grouped according to an index of clinical acromegalic activity also showed that IGF-I had fewer overlapping values than IGFBP-3 when patients with moderate or severe clinical activity were compared with subjects without clinical acromegalic activity.

Previous study has demonstrated that the decline in circulating IGF-I with age is more rapid than the decline in IGFBP-3[11]; the fact that GH secretion declines with increasing age,[15] together with the elevated IGF-I/IGFBP-3 ratio in acromegalic patients[11] in whom GH secretion is excessively high, suggested that serum IGF-I is more closely regulated than IGFBP-3 by GH. This could explain the smaller discriminative value of IGFBP-3 compared with IGF-I observed in the present study.

The ratio IGF-I/IGFBP-3 has been proposed as an index of free, biologically active IGF-I.[11] Juul et al[11] have shown that the ratio IGF-I/IGFBP-3 is significantly higher in acromegalics than in controls. They also demonstrated a significant correlation between this ratio and the urinary GH concentration or serum GH level.

On the other hand, Jørgensen et al[16] reported that the aforementioned ratio was similar when comparing active and successfully treated acromegalics and control subjects. In our study, the ratio IGF-I/IGFBP-3 in patients with acromegaly was generally higher than in controls. However, individual comparison of the ratio between patients with active disease and controls showed overlapping values similar to those found with determination of IGFBP-3.

The present investigation also found a significant relationship between the index of clinical activity of acromegaly and plasma IGF-I, serum IGFBP-3, or the IGF-I/IGFBP-3 ratio, which extended previous data on the relationship of plasma IGF-I with an index of clinical activity.[4]

In conclusion, the present data suggest that total plasma IGF-I levels may be more discriminatory in evaluation of acromegalic patients than serum IGFBP-3 levels alone or expressed as an IGF-I/IGFBP-3 ratio, but definitive conclusions in this regard would require analysis of a larger group of subjects.

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