Evaluation of plasma insulin-like growth factor binding protein-2 as a marker for adrenocortical tumors

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Abstract

Objective: Recent studies have pointed to the role of the IGF system in the pathogenesis of adrenocortical tumors, and it was shown recently that malignant adrenocortical tumors exhibit a high insulin-like growth factor binding protein (IGFBP)-2 content. Circulating markers specific for adrenocortical carcinoma are needed and the aim of this study was to evaluate plasma IGFBP-2 as a marker for these malignant tumors.

Methods: Plasma IGFBP-2 was determined in 51 patients referred to our institutions for adrenocortical tumors. Fifteen patients were in complete remission (group 1), eight patients had preoperative localized tumors (group 2) and 28 patients had metastatic tumors (group 3). Thirty-six healthy volunteers constituted a control group.

Results: There was no significant difference in plasma IGFBP-2 concentration between healthy controls and patients with complete remission or localized tumors. In contrast, patients with metastatic disease had significantly higher IGFBP-2 plasma levels than the control group ($P < 0.001$). IGFBP-2 levels in patients with metastatic disease were inversely correlated with survival ($R^2 = 0.308; P = 0.0026$). In patients with localized tumors, there was no correlation between plasma IGFBP-2 concentration and tumor size or histological features. Analysis of individual IGFBP-2 concentrations showed that five patients (17.8%) with metastatic tumors had normal IGFBP-2 levels and two patients (13.3%) in complete remission had high plasma IGFBP-2 levels. The influence of nutrition, hormone secretion and treatment on IGFBP-2 levels was examined. Nutritional status was evaluated by determining IGF-I levels and was found to be normal in 16 patients (61.5%) with high IGFBP-2 levels, suggesting that malnutrition was not responsible for the high IGFBP-2 concentrations in these patients. IGFBP-2 levels did not differ significantly according to tumor secretion or mitotane treatment. In a follow-up study, plasma IGFBP-2 concentration remained stable in patients with complete remission or stabilized disease and was a late marker of tumor progression in patients with progressive metastatic disease.

Conclusions: These results indicate that plasma IGFBP-2 is elevated in patients with malignant adrenocortical tumors and that the major factor affecting IGFBP-2 levels in these patients is tumor stage. However, plasma IGFBP-2 was less sensitive than expected for a tumor marker, which may limit its value in the diagnosis and follow-up of adrenocortical carcinoma.

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Introduction

Adrenocortical carcinoma is a rare tumor with an approximate annual incidence of two cases per million population (1). Its prognosis is poor, with fewer than 20% patients surviving 5 years after diagnosis (2). These tumors are often diagnosed at an advanced stage, at which treatment is only palliative (3). In small localized tumors, the main problem is distinguishing unambiguously between benign and malignant tumors as there are no absolute histologic criteria for malignancy.

Recent studies have identified various factors associated with the proliferation and transformation of adrenocortical tumor cells (1, 4). One of them, insulin-like growth factor-II (IGF-II) is strongly associated with adrenocortical malignancy. IGF-II has been shown to be involved in the proliferation of adrenocortical tumor cells in vitro, using the NCI H295R cell line derived from a human adrenocortical carcinoma (5). Furthermore,
human malignant adrenocortical tumors frequently exhibit dysregulation of the IGF system. These include an imprinting mistake of the 11p15 region in which the IGF-II gene is located, overexpression of IGF-II and its receptor, the type 1 IGF receptor, and a high content in IGF-binding protein-2 (IGFBP-2) (6–10).

These molecular markers are potential new tools for the diagnosis and prognosis of adrenocortical tumors (4). By contrast, there is no specific circulating marker available for adrenocortical carcinoma, which would help in identifying patients at high risk of malignancy before surgery. For instance, the concentration of IGF-II, which is overexpressed in malignant tumors, is not high in the plasma of patients suggesting a local role for this growth factor (7). Analysis of tumor secretions has suggested that adrenocortical carcinomas preferentially produce androgens and steroidogenic precursors (1, 11, 12). These secretory profiles may be of use in the follow-up of treated patients but they are neither specific nor sensitive markers of adrenocortical malignancy (2).

In vivo, the IGFs are bound with high affinity to IGF-binding proteins (IGFBPs). Six IGFBPs have been described to date, all of which locally modulate negatively or positively the effects of IGFs (13,14). We have previously shown that malignant adrenocortical tumors contain large amounts of one particular IGF-binding protein, IGFBP-2 (5, 10). The precise role of IGFBP-2 in adrenocortical tumorigenesis is presently unknown. However, similarly high levels of IGFBP-2 have been described in models of various tumors including lung carcinoma, prostate, ovarian and Wilms’ tumors, rhabdomyosarcoma and neuroblastoma (15–20). High levels of circulating IGFBP-2 have also been demonstrated in patients with these malignancies (16, 21–25). In patients with prostate carcinoma and ovarian tumors, serum IGFBP-2 has been shown to correlate with specific tumor markers (PSA and CA125 respectively) (24, 26). This suggests that circulating IGFBP-2 is related to tumor cell proliferation and might therefore be of clinical use for determining prognosis and in the follow-up of patients with these tumors.

In light of these observations and because malignant adrenocortical tumors have a high IGFBP-2 protein content, we hypothesized that circulating IGFBP-2 levels would be elevated in adrenocortical carcinoma. In the present study, we assessed the value of plasma IGFBP-2 (i) as a marker of malignancy in preoperative adrenocortical tumors and (ii) in the follow-up of patients with adrenocortical carcinoma.

Patients and methods

Patients

Fifty-one patients (20 males, 31 females; mean age, 43.4 ± 14.1 years; range, 18–78 years) followed in our institutions for adrenocortical tumors were included in this study. Tumors were defined according to histological criteria (Weiss score) and TNM classification (27). Localized tumors (stages I and II) were classified as benign (absence of all criteria for malignancy), suspect (Weiss score 1–3) or malignant (Weiss score >3) (8, 28). The characteristics of the patients are presented in Table 1. Patients were assigned to three groups according to the extent of the tumor after imaging evaluation (baseline X-rays, abdominal ultrasonography, computerized tomography (CT) scans of the abdomen and the chest).

Group 1 consisted of 15 patients in complete remission as defined by World Health Organization (WHO) criteria (29). All these patients had undergone successful surgery with complete removal of the tumor before this study. Group 2 consisted of eight preoperative patients with localized adrenocortical tumors (stage I or II). Staging of the tumor was confirmed by subsequent surgery and histological examination. Group 3 consisted of 28 patients with metastatic disease (liver and/or lung metastases, n = 28; bone metastases, n = 3). Twenty-five of these 28 patients (89%) had previously undergone surgery. For this group, survival was calculated from the time of IGFBP-2 determination.

As shown in Table 1, 28 of the 51 patients received at least one treatment during the study including mitotane and chemotherapy (3).

Several blood samples were available for 17 patients during a follow-up study (mean follow-up: 6.7 months; range, 2–14 months) (Table 1). For five patients (patients 30, 32, 41, 42 and 46; Table 1), comparisons between plasma IGFBP-2 levels and evolution of the disease was performed at various periods of the follow-up study (partial response to treatment, stabilized disease and progressive disease). For these 17 patients, evolution of the disease was determined after complete imaging evaluation according to WHO criteria. Stable disease was defined as a reduction of less than 50% or an increase of less than 25% in the sum of the products of the perpendicular diameters of all lesions with no evidence of new lesions between two IGFBP-2 measurements at least 4 weeks apart. A partial response to treatment was defined as a reduction of at least 50% in the sum of the products of the longest perpendicular diameters of all measurable lesions and the absence of new lesions. Progressive disease was defined as an increase greater than 25% in tumor size or the appearance of new lesions (29).

The control group consisted of 36 healthy volunteers (14 males, 22 females; mean age, 39 ± 16 years; range, 17–78 years) with no history of tumor, renal or hepatic disease, or malnutrition. There was no significant difference in age or sex between the control group and the group of patients.
Table 1 Characteristics of the 51 patients.

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<th>Tumor weight (g)</th>
<th>Tumor stage at time of study</th>
<th>Duration of remission (months)</th>
<th>Hormonal pattern(^b) at time of study</th>
<th>Treatment at time of study</th>
<th>Evolution of disease(^c) during follow-up study</th>
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\(a\): arbitrary numbers; \(b\): GC = glucocorticoid, A = androgens, E2 = estrogens, M = mineralocorticoid secretions, NS = non secreting; \(c\): CR = complete remission, PR = partial response, ST = stabilized disease, PD = progressive disease.
This study was performed in accordance with local ethical guidelines, with the informed consent of the patients.

**Methods**

Plasma samples were collected after overnight fasting and before treatment in patients receiving chemotherapy. All patients had normal liver and kidney function (plasma creatinine <110 μM).

Plasma IGFBP-2 was determined using an immunoassay kit from Diagnostic Systems Laboratories (Webster, TX, USA). The intra- and interassay coefficients of variation were 6.5 and 10.3% respectively. Normal IGFBP-2 values were defined as those between -2 and +2 standard deviation score (SDS) as calculated from the control group. For the follow-up study, changes in IGFBP-2 levels (ΔIGFBP-2) were calculated as follows: ΔIGFBP-2 = (IGFBP-2 level in sample 2 - IGFBP-2 level in sample 1)/IGFBP-2 level in sample 1 × 100.

Plasma IGF-I was determined using an immunoradiometric assay after an acid–ethanol extraction (Immunotech, Marseille, France). The intra- and interassays coefficients of variation were 7.2 and 12.4% respectively.

The hormone secretion profile included serum cortisol and 24 h urinary free cortisol, testosterone, dehydroepiandrosterone sulfate (SDHA), androstenedione, 17β-estradiol, aldosterone, 11-deoxycortisol and 11-deoxycorticosterone.

**Statistical analysis**

All analyses were performed using the Statview statistical package (Abacus Concepts, Inc., Berkeley, CA, USA). Data indicated in the text represent means ± standard deviation. Differences between the control group and the groups of patients were tested with the non-parametric Mann–Whitney U test. The correlation between IGFBP-2 and IGF-I or survival was evaluated by linear regression. Statistical significance was taken at P < 0.05.

**Results**

**IGFBP-2 levels in controls and patients with adrenocortical tumors**

Plasma IGFBP-2 concentration was determined in healthy controls and patients with adrenocortical tumors (Fig. 1). In the patient and control groups,
most subjects (90 and 85% respectively) were under
the age of 60 years, so plasma IGFBP-2 concentrations
did not vary significantly with age ([26] and data not
shown).

In healthy controls, mean IGFBP-2 plasma concen-
tration was $470 \pm 130$ ng/ml ($-2$ to $+2$ SDS: $210-$
$730$ ng/ml). Plasma IGFBP-2 levels did not differ
significantly between healthy controls and patients
with complete remission or localized tumors (group 1,
mean IGFBP-2 $= 612 \pm 265$ ng/ml; group 2, mean
IGFBP-2 $= 572 \pm 200$ ng/ml). In contrast, patients
with metastatic tumors (group 3) had significantly
higher levels of IGFBP-2 than the control group (mean
IGFBP-2 $= 1314 \pm 554$ ng/ml; $P < 0.001$). In group
3, plasma IGFBP-2 levels were found to be negatively
correlated with survival ($R^2 = 0.308$; $P = 0.002$;
mean survival $= 8.3 \pm 4.7$ months).

In the group of patients with localized tumors (group
2), preoperative IGFBP-2 levels did not correlate with
tumor weight or histological grade (Weiss score). Five
patients had tumors with histological features of
malignancy, but only one of them (patient 23, Table 1)
had high IGFBP-2 plasma levels before surgery. For this
patient, surgery confirmed the tumor stage (stage II) and
histological examination indicated a Weiss score of 5
including invasion of the tumor capsule.

Analysis of individual IGFBP-2 values showed that 5
of 28 (17.8%) patients with metastatic tumor dissemi-
nation had normal IGFBP-2 levels. Conversely, in the
group of patients in complete remission, 2 of 15
(13.3%) had high plasma levels of IGFBP-2. These two
patients (patients 3 and 11, Table 1) had no metastatic
spread at the time of the study as assessed by complete
imaging screening. Patient 11 had been successfully
treated for a localized malignant tumor (stage II) with a
follow-up period of 11 months and patient 3 for a
benign tumor with a follow-up period of 7 months.

**Factors associated with high plasma IGFBP-2
concentration**

Prolonged fasting and malnutrition may increase
circulating IGFBP-2 levels so it was important to
evaluate the nutritional status of each patient ([30],
[31]). Because IGF-I is a good nutritional marker, IGF-I
levels were determined for all patients ([32] (Fig. 2).
Plasma IGFBP-2 concentration was found to be
negatively correlated with IGF-I ($R^2 = 0.22$, $P =
0.0029$). However, 16 of 26 (61.5%) patients with
high IGFBP-2 levels had normal or high levels of IGF-I,
indicating a good nutritional status.

Hormone secretion was evaluated for all patients. In
patients with adrenocortical tumors (group 2 and 3;
$n = 36$), 16 had non-secreting tumors and 20 had
hormonally active tumors: 16 secreted glucocorticoid
with or without androgens, two secreted estrogens and
two secreted androgens only. No significant difference
in IGFBP-2 level was found between patients with non-
secreting tumors and those with hormonally active
tumors. In the same group of patients, there was no
significant difference in IGFBP-2 levels between
untreated patients ($n=14$) and those treated with
mitotane ($n = 22$).

**IGFBP-2 levels during follow-up in patients
with adrenocortical tumors**

Figure 3 shows the changes in IGFBP-2 levels in 17
patients for whom a follow-up study was performed.
(Table 1). Five of the six patients who remained in complete remission during the follow-up period had IGFBP-2 values consistently within the normal range (mean ΔIGFBP-2 = -8%). During the follow-up study, four patients had stable disease with corresponding stable IGFBP-2 levels (mean ΔIGFBP-2 = -5%) and two patients presented a partial response to treatment with a decrease in plasma IGFBP-2 concentration over the same period (ΔIGFBP-2 = -48 and -27%). In the group of patients with progressive disease (n = 11), IGFBP-2 levels correlated with the progression of the disease in six patients (mean ΔIGFBP-2 = +142%). For the other five patients, plasma IGFBP-2 concentration was within the normal range or remained stable despite the progression of metastasis (mean ΔIGFBP-2 = +13.7%).

**Discussion**

High levels of circulating IGFBP-2 have been reported in various malignancies (16, 21–26). We have previously shown that malignant adrenocortical tumors have a high IGFBP-2 protein content, suggesting that circulating IGFBP-2 is a potentially valuable tumor marker in adrenocortical carcinoma (10).

In this study, we found high plasma IGFBP-2 levels specifically in patients with malignant metastatic adrenocortical tumors. IGFBP-2 levels being negatively correlated with survival in these patients.

Several lines of evidence support the notion that in patients with adrenocortical carcinoma, circulating IGFBP-2 originates from the tumor. First, malignant adrenocortical tumors and the H295R cell line, which is derived from a human adrenocortical carcinoma, contain large amounts of IGFBP-2 protein (5, 10). Second, in the follow-up study, IGFBP-2 levels changed in accordance with tumor evolution in most of the situations studied. Third, human IGFBP-2 has been detected in the serum of nude mice bearing H295R tumor cell xenografts (33).

Several factors in addition to tumor load are known to affect the levels of circulating IGFBP-2 and may have increased IGFBP-2 concentration in our patients. None of them had renal or hepatic dysfunctions which may elevate IGFBP-2 levels (34, 35). IGFBP-2 levels increase with age (34) but in this study, most of the patients were under the age of 60 years and there is no significant difference in IGFBP-2 levels within this age range (26).

Malnutrition and cachexia are known to increase IGFBP-2 levels and occur frequently in patients with extensive tumors (30, 31). We found a negative correlation between plasma IGFBP-2 concentration and the concentration of the nutritional marker IGF-I. This suggests that malnutrition probably affected circulating IGFBP-2 levels in some of the patients with adrenocortical tumors, mostly those with advanced metastatic disease.
However, 61.5% of the patients with high plasma IGFBP-2 levels had normal or high IGF-I levels reflecting a good nutritional status. This suggests that malnutrition is not the major factor responsible for high plasma IGFBP-2 levels in these patients.

Acute glucocorticoid administration has been shown to decrease IGFBP-2 levels (36). However, high levels of serum IGFBP-2 have been measured in patients with Cushing’s disease ((37) and our unpublished results). If long-term glucocorticoid secretion is responsible for elevated circulating IGFBP-2 in these patients, it may also account for increased IGFBP-2 levels in patients with glucocorticoid-secreting adrenocortical tumors. However, in this study, there was no difference in plasma IGFBP-2 level between patients with glucocorticoid-secreting tumors and patients with non-secreting tumors, suggesting that glucocorticoid secretion by adrenocortical tumors had no major effect on circulating IGFBP-2. Similarly, mitotane treatment, which inhibits steroidogenesis, did not seem to affect IGFBP-2 levels.

Altogether, these observations indicate that tumor stage is the predominant factor influencing IGFBP-2 levels in patients with adrenocortical carcinoma.

Another question addressed in this study was that of the value of plasma IGFBP-2 for diagnosing malignancy in preoperative localized tumors and for the follow-up of patients.

In the small group of patients with localized adrenocortical tumors, five patients had limited tumors with histological features of malignancy but only one patient had high plasma IGFBP-2 levels. We found no correlation between tumor weight or histological grade and IGFBP-2 levels in this group. Thus, plasma IGFBP-2 cannot be regarded as a marker of malignancy in localized adrenocortical tumors.

In patients with metastatic disease, we found a significant negative correlation between IGFBP-2 levels and survival suggesting that, in these patients, circulating IGFBP-2 levels reflected tumor extension. However, analysis of individual IGFBP-2 values showed that five patients (17.8%) with metastatic tumors had normal IGFBP-2 levels. Four of these five patients were considered to have stabilized disease under mitotane treatment or chemotherapy at the time of the study. Interestingly, these five patients had a significant longer survival than metastatic patients with high IGFBP-2 levels (12.4 months versus 7.4 months; P = 0.03). In the follow-up study, 5 of 11 patients (45%) had no increase in IGFBP-2 levels despite progression of the disease. Thus, although circulating IGFBP-2 concentration correlates with tumor extension, it has a poor sensitivity as a tumor marker, precluding its use in the follow-up of patients with adrenocortical carcinoma.

These conclusions are consistent with results obtained for other malignancies. In a study of 50 children with acute lymphoblastic leukemia (ALL), Wex et al. found that 40% of the patients had normal IGFBP-2 levels at the time of diagnosis, suggesting low sensitivity of circulating IGFBP-2 concentration (38). Similarly, Mohnike et al. studied four patients with ALL relapse and found only one patient with high serum IGFBP-2 levels (25). Ho et al. compared serum IGFBP-2 and PSA levels in patients with prostate cancer and concluded that serum IGFBP-2 was less sensitive as a tumor marker than PSA (26). Similar conclusions were drawn from a study on ovarian tumors (24).

In the group of patients with complete remission, two patients (13.3%) had high plasma IGFBP-2 levels. The reasons for the high IGFBP-2 levels in these two patients are unclear. Both had good nutritional status. One patient (patient 11; Table 1) had been treated successfully for a malignant tumor with no sign of recurrence at the time of the study. The other patient (patient 3; Table 1) had high IGFBP-2 levels (1450 ng/ml) despite complete removal of a benign tumor. This patient had severe hypertension treated with several antihypertensive drugs, which may have interfered with hepatic IGFBP-2 production or clearance.

In conclusion, this study indicates that tumor stage is the predominant factor influencing IGFBP-2 levels in patients with adrenocortical carcinoma, and that, in these patients, increased plasma IGFBP-2 levels are associated with a poor survival. However, plasma IGFBP-2 is less sensitive than expected for a tumor marker, which may limit its value in the diagnosis and follow-up of patients with adrenocortical carcinoma.

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