# Is There a Role for the IGF System and Epidermal Growth Factor (EGF) in the Pathogenesis of Adrenocortical Adenomas? A Preliminary Case-Control Study

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## Summary

Adrenal incidentalomas (AI) are very common and mostly they are non-functioning adenomas (NFA). NFAs are often associated with insulin resistance and metabolic syndrome. Several biomarkers, including certain growth factors, may participate in the pathogenesis of metabolic changes in patients with adrenal adenomas. Patients with NFA and age-matched control subjects were enrolled in the study. Data on age, gender, presence of metabolic syndrome or its components were obtained for each subject. Blood samples were obtained and glycemia, insulinemia, lipid profile, and selected growth factor levels were measured. Forty-three patients with NFA and 40 controls were included in the study. Differences were not found in the metabolic syndrome and its components prevalence or in the biochemical profile between patients and the control group. Significant differences were noticed in the levels of IGF1, IGF2, and IGFBP3 (p=0.016, p=0.005, p=0.004, respectively), but there were no differences in VEGF or EGF concentrations. In NFA patients, an association between glycemia and EGF levels was present (p=0.026). No significant correlations between tumor size and insulin or growth factor concentrations were present in AI patients. Significantly higher serum IGF1, IGF2, and IGFBP3 concentrations in NFA patients may support the role of the IGF axis in the pathogenesis of adrenocortical lesions. No correlation between IGFs or IGFBP3 and parameters of glucose or lipid metabolism was found. Present results may support the role of the growth hormone axis rather than hyperinsulinemia and insulin resistance in the pathogenesis of adrenocortical adenomas.

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## Introduction

Adrenal incidentalomas (AI) are among the most common tumors in humans with an estimated prevalence of 1 – 8 %. The vast majority of these tumors are benign adrenocortical adenomas (ACA, Fassnacht *et al.* 2016). Some of them produce hormones but, in most cases, they are non-functioning adenomas (NFA, Khan 2019). Although patients with NFA do not show any overt physical signs of excessive hormonal production, they are often insulin resistant and have metabolic syndrome (Muscogiuri *et al.* 2017). Currently, it is not completely clear if ACA development results from primary insulin resistance and compensatory hyperinsulinemia, or insulin resistance develops secondly due to the slight cortisol hypersecretion by AI (Papanastasiou *et al.* 2017, Di Dalmazi 2017). Recent studies showed that the degree of insulin resistance directly correlates with the AI size (Delibasi *et al.* 2015). This allows hypothesizing that hyperinsulinemia could have mitogenic and mutagenic

effects on the adrenal cortex through the activation of insulin (insulin resistance) and insulin-like growth factor (IGF) system. Several biomarkers have been identified to be involved in the pathogenesis of obesity and insulin resistance in humans and also in patients with ACA. Besides various proinflammatory cytokines and adipocytokines, IGF1and IGF2 are the most studied insulin-like peptides regulating a large number of important physiological processes (Livingstone and Borai 2014, Anderlová *et al.* 2019). IGF1 has multiple biological effects, such as the promotion of cell growth and proliferation, as well as regulation of fuel metabolism. It has structural and functional similarities to insulin and plays a role in glucose homeostasis together with insulin growth factor binding protein 3 (IGFBP3), which binds the majority of circulating IGF1 (Aneke-Nash *et al.* 2017, Xie *et al.* 2015). IGF1 is known to promote cancer development by inhibiting apoptosis and stimulating cell proliferation. Epidemiological studies have reported a positive association between circulating IGF1 levels and various tumors, especially breast, colon, prostatic and adrenocortical cancer (ACC) (Shanmugalingam *et al.* 2016). IGF2 is a widely expressed mitogenic peptide primarily regulating fetal development and differentiation, but its role in adults is little understood (Kadakia and Josefson, 2016). IGF2 levels have been found to be associated with metabolic parameters such as insulin resistance or bodyweight (Murphy 2003). In adrenocortical tumors, especially ACC, *IGF2* is the most frequently and strongly overexpressed gene (Kool *et al.* 2015).

Epidermal growth factor (EGF) is a small protein of 6 kDa containing 53 amino acids comprising three disulfide bridges. It is characterized by mitogenic activity and so can be involved in the processes of cell growth and tumorigenesis. In fact, EGF is a protein contained in a network of growth factors and receptors that controls the growth and division of cells (Yarden 2001). EGF receptor (EGFR) has been demonstrated to have a pivotal role in tumorigenesis, with many human solid tumors overexpressing EGFR, including colon, breast, lung cancer, and ACC (Adam *et al.* 2010). Studies showed that EGFR is overexpressed in more than three-quarters of ACC cases. Rarely, its expression was demonstrated also in ACA (Adam *et al.* 2010).

From the clinical point of view, components of the IGF system are at a first-place considered as diagnostic serum and/or tissue biomarkers of a given cancer, they also serve as prognostic factors and attractive target of modern anti-tumor therapies. There are only a few studies evaluating serum levels of IGFs, EGF, and VEGF and assessing their relationship to ACA (Kasprzak *et al.* 2017). The objective of the present study is to assess and compare metabolic parameters, IGFs (IGF-1, IGF-2, and IGFBP-3), EGF, and vascular endothelial growth factor (VEGF) in patients with NFA and control subjects. It also aims to determine the relationship between growth factors and insulin or serum cortisol in the group of patients with NFA.

## Patients and Methods

Patients with AI sized >1 cm in the longest dimension found on computed tomography (CT) examination performed for other reasons were selected for the present study. From each patient, we obtained the data about tumor size and side location. AIs had to have characteristics of adenoma (homogenous low density mass) on computed tomography (CT) images or adenoma was confirmed histologically after adrenalectomy in all patients. Adrenalectomy was indicated in patients with tumor size >4 cm or if slight cortisol overproduction was confirmed (low ACTH and/or DHEA-S levels and serum cortisol concentration ≤60 nmol/l after a 1-mg overnight dexamethasone suppression test). Patients with pheochromocytoma, overt Cushing syndrome, or primary hyperaldosteronism, as well as patients with acromegaly, were excluded from the study.

Control group was selected from the age-matched general population, exclusion criteria included severe heart failure, liver and/or kidney disease acromegaly and any adrenoal pathology. All control subjects had to have a negative ultrasound (US), CT, or magnetic resonance (MRI) imaging of kidneys and adrenals. The CT characteristics of adenoma included a tumor density lower than 18 HU, relative washout >50 % over 10-15 minutes and well margination.

Data on age, gender, presence of hypertension, diabetes mellitus, dyslipidemia, and metabolic syndrome were obtained for each patient.

All subjects involved in the study signed a written informed consent and the study was approved by the Ethical Committee of the University Hospital in Košice, Slovakia.

*Ethics approval*

The study was approved by the Ethical Committee of the University Hospital in Košice,

Slovakia. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

*Laboratory tests*

Venous blood was drawn in all subjects in the morning after a 12-hour fasting period into polyethylene tubes without an anticoagulation agent (for serum) and into tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant. All samples were immediately centrifuged at 4 °C and both serum and plasma were stored at -80 °C until they were processed.

Serum glucose levels as well as lipid profile (total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides (TAG) were analyzed routinely using an autoanalyzer (Roche Diagnostics GmbH). Insulin concentrations were determined from serum by immunoradiometric assay (Beckman Coulter, Inc.). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by the equation: HOMA-IR= (glucose × insulin)/22.5.

Serum cortisol and ACTH were measured from the same morning sample using RIA kit Immunotech Beckmann (Czech Republic) and RIA kit BRAHMS (France), respectively.

The levels of VEGF and EGF were determined in blood serum (100 µl) by Cytokine & Growth Factors High Sensitivity Array (Cat. No. EV3623, Randox Laboratories Ltd. Crumlin, UK) by Randox Evidence Investigator Analyzator (Serial No: EI-15354, Randox Laboratories Ltd. Crumlin, UK) simultaneously. Assay sensitivity varied from 0.12 pg/l to 2.12 pg/l (https://www.eposters.net/pdfs/multiplexed-measurement-of-cytokines-in-saliva-with.pdf) depending on specific cytokine or growth factor marker. The repeatability of the assay for individual cytokine or growth factor marker was determined using the quality controls provided with the CTK HS kit. The levels of IGF1, IGF2, and IGFBP3 were determined using immunoradiometric assays (Beckman Coulter, Inc).

*Statistical analysis*

SPSS Statistics Faculty Pack (IBM, USA) was used for statistical analysis. Categorical variables were summarized as frequency counts and percentages. Continuous variables were summarized as the median and range. Comparisons of percentages in cross-tabulated tables to assess independence or association of factors used Fisher's exact test for both simple 2 x 2 contingency tables and those with more rows and/or columns. This test assumes that neither the rows nor columns have an inherent ordering, as was the case in our applications. Wilcoxon Rank Sum test was used for numeric value comparisons. Linear regression analysis was used to assess correlations between variables. Multiple logistic regression analysis was performed to determine which variables were associated with Ais. Values were considered to be statistically significant at p≤0.05. All reported p-values are two-sided.

# Results

*Patients characteristics*

Forty-three patients (14 [32.56 %] males and 29 [67.44 %] females) with AI were included in the

**Table 1.** Patients´ and control group characteristics and prevalence of metabolic syndrome and its components

|  | **Patients** | **Controls** | **p-value** |
|---|---|---|---|
| *Total (N)* | 43 | 40 | |
| *Males (N,%)* | 14 (32.56 %) | 19 (47.5 %) | 0.17 |
| *Females (N,%)* | 29 (67.44 %) | 21 (52.5 %) | 0.17 |
| *Age (median, range)* | 66.81 (44.23–82.72) | 67.7 (26.52–91.44) | 0.69 |
| *Hypertension (N,%)* | 35 (81.4 %) | 29 (72.5 %) | 0.34 |
| *T2DM (N,%)* | 9 (20.93 %) | 18 (45.0 %) | 0.06 |
| *Dyslipidemia (N,%)* | 13 (30.23 %) | 19 (47.5 %) | 0.11 |
| *Metabolic syndrome (N,%)* | 7 (16.28 %) | 6 (15.0 %) | 0.87 |
| *Tumor size (mm, median and range)* | 20 (9-110) | – | – |

N - number, T2DM - type 2 diabetes mellitus

present prospective case-control study. In all patients, AI had characteristic CT image of adenoma or adenoma was confirmed histologically after adrenalectomy. The median age of patients was 66.8 (range 44 – 83) years. The age-matched control group consisted of 40 (19 [47.5 %] males, 21 [52.5 %] females) subjects with median age of 67.7 (range 26 – 91) years (Table 1) and

negative imaging findings on adrenal glands. There were no significant differences in the prevalence of type 2 diabetes mellitus (T2DM), metabolic syndrome, hypertension, or dyslipidemia between patients and the control group (p=0.06, p=0.87, p=0.34, p=0.11, respectively, Table 1).

**Table 2.** Biochemical characteristics of AI patients and controls

| Parameter | Patients (median, range) | Controls (median, range) | p-value |
|---|---|---|---|
| Glucose (mmol/l) | 5.35 (3.8-10.2) | 5.6 (3.1-16) | 0.459 |
| Insulin (mIU/l) | 14.6 (0.75-78.7) | 9.7 (0-175) | 0.294 |
| Cortisol (AM, nmol/l) | 389 (44.5-788.6) | – | – |
| ACTH (pmol/l) | 3.37 (0.46-11.33) | – | – |
| HOMA-IR | 3.94 (0.16-30.43) | 2.5 (0-38.11) | 0.685 |
| Total cholesterol (mmol/l) | 5.27 (2.92-7.72) | 4.57 (1.93-7.01) | 0.040 |
| LDL cholesterol (mmol/l) | 3.39 (1.27-5.44) | 2.94 (1.29-5.36) | 0.156 |
| HDL cholesterol (mmol/l) | 1.3 (0.88-1.84) | 1.095 (0.41-2.39) | 0.047 |
| TAG (mmol/l) | 1.4 (0.72-2.73) | 1.45 (0.57-3.69) | 0.503 |
| VEGF (pg/ml) | 155.71 (24.45-483.93) | 141.24 (22.8-713.02) | 0.529 |
| EGF (pg/ml) | 35.02 (1.5-390.69) | 45.075 (3.7-224.03) | 0.307 |
| IGF1 (ng/ml) | 157.17 (47.33-288.41) | 100.84 (24.64-218.38) | 0.016 |
| IGF2 (ng/ml) | 666.12 (338.16-1298.7) | 529.2 (233.61-1243.21) | 0.005 |
| IGFBP3 (ng/ml) | 1874.42 (1009.17-3178.72) | 1305.56 (278.09-3444.35) | 0.004 |

EGF - epidermal growth factor, HOMA-IR - homeostatic model assessment of insulin resistance, IGF - insulin-like growth factor, IGFBP3 - IGF binding protein 3, HDL - high-density lipoproteins, LDL - low-density lipoproteins, TAG - triacylglycerols, VEGF - vascular endothelial growth factor
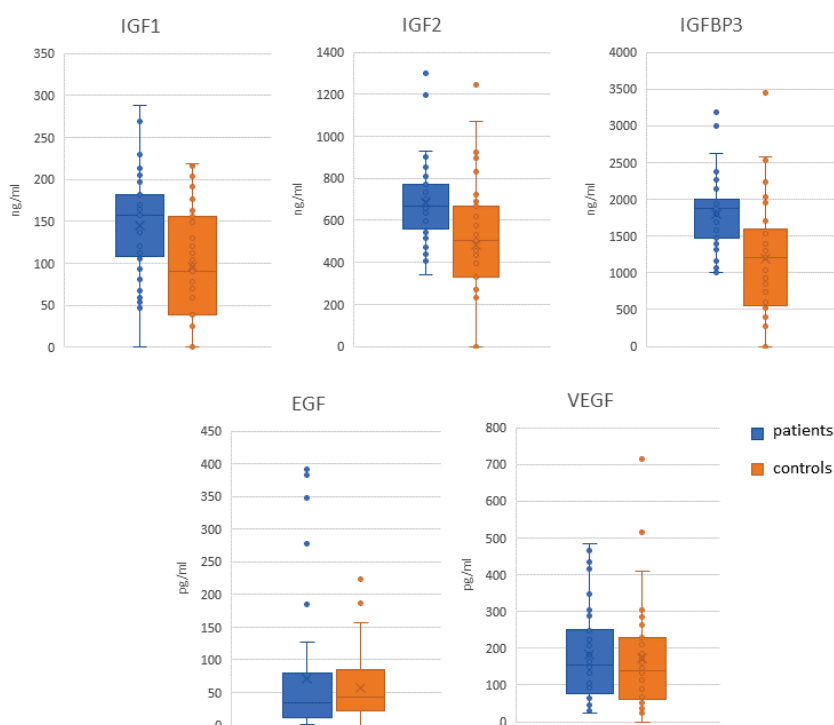


**Fig. 1.** Studied growth factors serum concentrations in patients and controls. EGF - epidermal growth factor, IGF - insulin-like growth factor, IGFBP3 - IGF binding protein 3, VEGF - vascular endothelial growth factor

*Biochemical results*

There were no significant differences in glycemia, insulinemia, insulin resistance index (HOMA-IR), total and LDL cholesterol between AI patients and control group subjects. However, HDL levels were lower by 0.205 mmol/l in the control group when compared to AI patients (1.095 vs. 1.3 mmol/l), which was on the border of statistical significance (p=0.047, Table 2).

When looking at the growth factor and interleukin levels in the AI patients and control group, no significant differences were found in the levels of VEGF and EGF (p=0.529 and p=0.307, respectively, Fig. 1. IGF1, IGF2 as well as IGFBP3 concentrations were significantly higher in the group of patients with AI when compared to the control group (p=0.016, p=0.005, p=0.004, respectively, Fig. 1).

There was an indirect relationship between glycemia and EGF levels in patients with AI (p=0.026). No correlation was found between serum morning cortisol levels and growth factor concentrations (Table 3).

No significant correlation between adrenal tumor size and concentration of insulin (p=0.844), IGF1 (p=0.570), IGF2 (p=0.974), IGFBP3 (p=0.709), VEGF (p=0.448), or EGF (p=0.074) was found in patients with AI (Table 3).

Using logistic regression analysis, no association between AIs and growth factors (IGF1, IGF2, IGFBP3, VEGF, and EGF) was found (Table 4). In addition, no association between subclinical hypercortisolism and growth factors has been detected in this study.

**Table 3.** Correlations between tumor size or serum cortisol levels and metabolic variables and growth factors in patients with AI

|  | Size of tumor (p-value) | Serum cortisol (p-value) |
| --- | --- | --- |
| *Glucose* | 0.191 | 0.846 |
| *Insulin* | 0.844 | 0.739 |
| *VEGF* | 0.448 | 0.301 |
| *EGF* | 0.07 | 0.942 |
| *IGF1* | 0.570 | 0.764 |
| *IGF2* | 0.975 | 0.301 |
| *IGFBP3* | 0.709 | 0.672 |

EGF - epidermal growth factor, IGF - insulin-like growth factor, IGFBP3 - IGF binding protein 3, VEGF - vascular endothelial growth factor

**Table 4.** Association between AI and growth factors

|  | OR (95 % CI) | p-value |
| --- | --- | --- |
| *(Intercept)* | 0.182 (-3.657–0.246) | 0.087 |
| *VEGF (pg/ml)* | 1.000 (-0.004–0.004) | 0.920 |
| *EGF (pg/ml)* | 1.001 (-0.005–0.008) | 0.753 |
| *IGF1 (ng/ml)* | 1.004 (-0.008–0.016) | 0.517 |
| *IGF2 (ng/ml)* | 1.001 (-0.004–0.005) | 0.710 |
| *IGFBP (ng/ml)* | 1.001 (-0.001–0.002) | 0.559 |

CI - confidence interval, EGF - epidermal growth factor, IGF - insulin-like growth factor, IGFBP3 - IGF binding protein 3, OR - Odds ratio, VEGF - vascular endothelial growth factor

## Discussion

In the present study, we examined the serum concentrations of IGFs, IGFBP-3, EGF, and VEGF in patients with AIs with typical CT signs of benign adenomas and age-matched control subjects with normal radiological finding on both adrenal glands. Although patients with ACA did not differ in the prevalence of metabolic syndrome from controls, they had significantly higher serum IGF1 and IGF2 as well as IGFBP3.

It is well known that AIs are frequently accompanied by metabolic syndrome or its components and with increased cardiovascular risk (Elhassan *et al.* 2019, Di Dalmazi 2019). Several studies reported that non-functional AIs can be associated with high levels of adipocytokines such as leptin and resistin, which affect insulin resistance and cardiovascular risk factors (Ermetici *et al.* 2007, Akkus *et al.* 2019). However, data on the serum levels of growth factors in patients with pathological adrenal masses are poor. The IGF system appears to be important in the regulation of adrenal growth and hormone synthesis (Altieri *et al.* 2016). According to some studies, this system also plays a crucial role in cardiovascular risk and metabolic syndrome (Livingstone and Borai 2014, Desai and Patel 2015, Obradovic *et al.* 2019, Harada *et al.* 2019). IGF1 is an anabolic growth hormone, responsible for cell growth, differentiation, proliferation, and survival (Desai and Patel 2015). Some studies confirmed its association with early tumorigenesis in animals and human (Wlodarczyk *et al.* 2019, Wlodarczyk *et al.* 2018). This relationship has been confirmed in the breast, pancreatic, and colorectal cancers as well as adrenocortical carcinoma (ACC) (Shanmugalingam *et al.* 2016). Many studies demonstrated higher expression of IGF1 and IGF2

receptors in ACC and larger adenomas, indicating a possible role of these growth factors in adrenal tumorigenesis (Altieri *et al.* 2019, Schmitt *et al.* 2006). Former study of Faical *et al.* (1998) found that adrenal adenomas and carcinomas had over 50 % of IGF1 positive cells in 64 % and 83 % of the tissue samples, respectively. The size of the tumors with 50 % of IGF1 positive cells compared with those with less than 50 % was, on average, greater, but no statistical differences between IGF1 cell positivity and the corresponding clinical syndrome were observed (Faical *et al.* 1998). Unfortunately, there are poor data on the serum levels of IGFs in patients with ACA. In the recent study of Bahadir *et al.* (2018), serum IGF1 levels were significantly higher in patients with NFA in comparison to controls (p=0.001), while HOMA-IR was similar in both groups. This finding is in agreement with the results of our study and can indicate an association between adrenal masses and IGF1. This is supported by the fact that patients with acromegaly and increased serum IGF1 concentrations demonstrated a significantly higher prevalence of pathologic adrenal masses especially ACCs (Jawiarczyk-Przybylowska *et al.* 2019, Terzolo *et al.* 2000). It remains to be recognized whether the growth hormone stimulatory effect or effect of hyperinsulinemia and insulin resistance are the leading factors for ACAs development.

The impact of IGF2 in ACC has been evaluated in some immunohistochemical and clinical studies (Kasprzak *et al.* 2017, Altieri *et al.* 2019, Guillaud-Bataille *et al.* 2014). Although IGF2 receptor overexpression is observed in the vast majority of ACCs and some larger ACAs, being a very helpful predictor for malignancy in adrenal tumors, the results of studies determining serum IGF2 levels are conflicting. In the study of Patel *et al.* (2014) IGF2 levels were not elevated in the serum of patients with ACC compared to those with benign adenomas and healthy volunteers. There were no significant differences in the serum IGF2 concentrations between controls and ACA which is in discordance with our study. Unfortunately, we did not find other clinical studies determining the serum levels of IGF2 in patients with ACA. However, serum IGF2 concentrations were examined in many studies in patients with metabolic syndrome. Some of these studies found its increase in obesity and a positive correlation with BMI (Buchanan *et al.* 2001). On the other side, recent studies demonstrated lower levels of this peptide in patients with metabolic syndrome (Pouriamehr *et al.* 2019). Although the prevalence of metabolic syndrome or its components

was similar in patients and controls in our study, increased levels of IGF2 suggest its possible role in the etiopathogenesis of ACAs together with IGF1.

However, the activity of the whole IGF system does not need to reflect the serum IGF1 and IGF2 concentrations, since the higher levels of IGFBP3 will presumably bind IGF in excess. Secondly, increased IGFs serum concentrations do not accurately reflect the paracrine IGF system activity in the adrenal tissue.

As IGFBPs modify IGF bioactivity, we investigated serum IGFBP3 in patients with ACA and controls. Based on recent data it has been suggested that both serum IGF1 and IGFBP3 are related to future risk of malignant neoplasms (Adachi *et al.* 2020). In our study serum levels of IGFBP3 were significantly higher in patients with ACA as compared with the control group. Currently, there are no clinical studies determining IGFBP3 in the serum of patients with adrenal adenomas. A former experimental study of Ilvesmäki et al. (1998) showed that IGFBP3 *mRNA* is very low in normal adrenals. In adrenal tumors and hyperplastic adrenal tissue, IGFBP3 *mRNA* expression was usually higher than in normal adrenal glands which might support our results. In the mentioned study, IGFBP3 *mRNA* expression was also higher in ACAs with cortisol overproduction, whereas no relationship between IGF1, IGF2, IGFBP3, and serum ACTH or cortisol concentration was found.

In the present study, we did not demonstrate significant differences in the serum EGF and VEGF levels among patients with ACA and controls. There was neither significant correlation of these growth factors with the size of the ACA nor with the serum cortisol. Several studies have reported EGF receptor overexpression in ACCs but not in ACAs, implying that the EGF receptor could potentially be used as a marker for the differential diagnosis of these two tumor types (Adam *et al.* 2010, Zhang *et al.* 2014, Angelousi *et al.* 2018). In the literature, there are no data concerning the serum concentrations of EGF in patients with ACA, and the data on the role of VEGF in the pathogenesis of ACA is poor. Previous studies did not demonstrate any differences in the serum VEGF concentrations between AIs and controls, although patients with AIs had significantly higher concentrations of VEGF receptor-2 than healthy subjects (Qin *et al.* 2013, Foltyn *et al.* 2012). To the best of our knowledge, this is the first study assessing together serum IGFs, EGF, and VEGF in patients with benign adrenal tumors in comparison to controls. Based on the results presented in this study it is

very difficult to postulate that IGF1, IGF2, or IGFBP3 may serve as biomarkers for ACAs or tumors in general and further clinical studies are required. Also, the growth factors in our study were measured by immunoradiometric assay or cytokine and growth factors high sensitivity array, which offer the most precise results.

Undoubtedly, our study has some limitations. The main constraint is a relatively small group of patients with AIs. Despite this fact, the statistical analysis and results allow postulating relevant conclusions that have not yet been published in the literature.

## Conclusion

In summary, we found that patients with ACAs have significantly higher serum concentrations of IGF1, IGF2, and IGFBP3 when compared to age and metabolically matched control subjects, indicating a possible role of the IGF axis in the pathogenesis of adrenocortical lesions. Serum IGFs and IGFBP3 did not correlate with the size of the ACAs. The present study did not show any correlation between IGFs or IGFBP3 and parameters of glucose or lipid metabolism. These results may support the hypothesis that the growth hormone axis rather than hyperinsulinemia and insulin resistance may be involved in the pathogenesis of ACAs.

## Conflict of Interest
There is no conflict of interest.

## Acknowledgements

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