Evaluation of the activity of arginase and some biochemical parameters in sera of patients with acromegaly

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Abstract

The objective of the present study was to study the effect of increasing Growth Hormone (GH) on arginase activity in sera of Iraqi acromegaly (ACRO) patients. Certain Vital biochemical parameters were measured such as Growth Hormone (GH), Insulin-Like Growth Factor-1 (IGF-1), Fasting Serum glucose (FSG), Urea, Total Cholesterol (TC), Triglycerides (TG), High-density lipoprotein-cholesterol (HDL-C), Low-density lipoprotein-cholesterol (LDL-C), and very low-density lipoprotein-cholesterol (VLDL-C). Eighty people between the ages ranged between of 25 and 65 were involved in this study, 40 of them were ACRO Iraqi patients and the remaining 40 were healthy controls. All participants were matched in age and sex and the body mass index (BMI) were calculated for each group. Arginase activity was reduced in ACRO patients significantly (p<0.05) compared to controls, also the HDL-C levels were reduced significantly p<0.05, while the levels of GH, IGF-1, TC, TG, LDL-C, and VLDL-C were elevated significantly p<0.05. There were no significant differences in the levels of FSG and urea between the patients and controls p>0.05. Results of the present study have revealed that patients with ACRO have significantly lower levels of serum arginase activity and not significantly lower levels of serum urea, which means their bodies retain more nitrogen compounds for use in building processes.

Keywords: Arginase, Acromegaly, Growth hormone, Insulin-Like Growth Factor-1, Lipid Profile.

Introduction

Acromegaly (ACRO) is a rare, complex hormonal syndrome classified as a chronic disorder which is characterized by elevated Growth hormone (GH), therefore Insulin-Like Growth Factor-1 (IGF-1) commonly caused by a GH-secreting pituitary adenoma. It induces multisystem impacts, especially in the osteoarticular system, muscles, brain, heart and blood vessels, respiratory and hematopoietic system, kidneys, liver, pancreas, thyroid, adipose tissue, and metabolic system. The ACRO also causes sexual dysfunction. This pathology occurs on average 40 to 70 people per million. The ACRO has a significant diagnostic delay that is linked to a higher risk of complications and deteriorating psychosocial conditions. The GH is essential for normal postnatal growth, it also plays a role in metabolism, muscle, bone, and lung homeostasis, reproduction, age-related physiological and pathological changes, immune response, chemotherapeutic resistance, and neoplastic development. The GH stimulates the formation of bones and modulates the metabolism of...
fats, carbohydrates, nitrogen, and minerals as well as electrolyte balance. The effects of GH can be both anabolic and catabolic. The IGF-1 mediates the majority of GH's anabolic actions. On the other hand, prolonged fasting results in a rise in GH without an elevation in IGF-1. In addition, GH enhances the ability of the liver and kidney to produce glucose via gluconeogenesis and glycogenolysis. The hormone-sensitive lipase is activated by GH, mainly in visceral adipose tissue, which causes free fatty acids (FFA) to flow from adipose tissue to the bloodstream.

The IGF-1, is a key growth factor that regulates both anabolic and catabolic pathways in skeletal muscle. Alteration in IGF-1 signaling in muscle tissue can have a significant impact on myofiber growth and function. Also, IGF-1 can influence protein synthesis as well as protein breakdown processes. Since IGF-1 promotes a mitogenic response and suppresses cell death (apoptosis) in a diverse cell range, potentially leading to cancer, despite its importance in cell survival. The metabolism and proliferation are significantly impacted by IGF-1. Its long-term action is thought to be similar to a growth factor, whereas its short-term impact is similar to insulin.

L-arginase (Arg) (EC 3.5.3.1) catalyzes, the last step in the urea cycle, is a binuclear manganese metalloenzyme. The enzyme hydrolyzes L-arginine to L-ornithine and urea, it exists in two isoforms in mammals, arginase 1 (Arg1) or liver arginase and arginase 2 (Arg2) or kidney arginase. The Arg is involved in the metabolism of L-arginine, the formation of nitric oxide, and a variety of signaling pathways, either independently or independently from its L-arginine urea hydrolase activity.

The balance between Arg and Nitric Oxide Synthesis (NOS) may be disrupted by a number of pathological conditions, which would disrupt the organism's homeostasis and function. A number of pathophysiological conditions, including cardiovascular, immune-mediated, tumor-producing, and neurodegenerative illnesses, are linked to elevated arginase activity. As far as we know there are no studies about the role of Arg activity in ACRO patients. Rising in Arg activity levels can be viewed as a sign of diabetes. Currently, Arginase inhibitors are regarded as promising therapies for the treatment of a variety of pathologies. The aim of this study was to study the effect of increasing GH on arginase activity in sera of Iraqi ACRO patients and compare it with a control group.

Materials and Methods

Patients and Control

From July to October 2022, 80 individuals ranging in age from 25 to 65 were included in this study. At the national diabetes center of Mustansiriyah University, in Baghdad, forty ACRO Iraqi patients were involved, and forty healthy individuals (controls), were matched in age and sex. For each group, BMI was computed.

Samples

To separate the serum, 10 ml of blood was collected via venipuncture and transferred into a gel tube. The blood sample was centrifuged to separate the serum at a speed of 3000 rpm for 10 min. The serum was then separated into four Eppendorf tubes and stored at -20 °C until testing. Samples of ACRO patients with diabetes were excluded.

Sample Analysis

Using Porembksa's method, arginase activity in serum was examined manually. A solid-phase ELISA kit provided by DRG Company / Germany was used to assess human GH and IGF-1 in order to quantify their serum concentrations. The Fasting serum glucose (FSG), urea, Total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL-C) were measured by using the Kenza240 TX Biolabo kit.

Statistical analysis

Mean ± SD was used to express the data. Independent-Samples student's t-test and correlation were employed to evaluate the relationship between the data using The SPSS version 26. P-values greater than 0.05, equal or less than 0.05, and less than 0.001 were regarded as non-significant (N.S), significant (S), and highly significant (H.S), respectively. GraphPad Prism 8 was used for drawing the graphs.
Results and Discussion

Table 1 shows there were no statistically significant $p > 0.05$ differences in age between the control group $44.69 \pm 10.30$ years and patients with ACRO $46.35 \pm 9.25$ years. The body mass index (BMI) value was found to be highly significant $p < 0.001$ in patients with ACRO $33.41 \pm 3.64$ kg/m$^2$ compared to control group $24.31 \pm 2.05$ kg/m$^2$.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (N = 40)</th>
<th>ACRO (N = 40)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yr</td>
<td>$44.69 \pm 10.30$</td>
<td>$46.35 \pm 9.25$</td>
<td>N.S</td>
</tr>
<tr>
<td>BMI kg/m$^2$</td>
<td>$24.31 \pm 2.05$</td>
<td>$33.41 \pm 3.64$</td>
<td>H.S</td>
</tr>
</tbody>
</table>

Abbreviations: ACRO, Acromegaly; BMI, Body Mass Index. $p$-value $>0.05$ is non-significant (N.S), $p$-value $\leq 0.05$ is significant (S), $p$-value $<0.001$ is highly significant (H.S).

The increase in BMI among ACRO patients was not brought on by obesity but rather by increased bone mineral density. A recent study has reported that compared to controls, BMI in ACRO patients was significantly increased, which agrees with the finding of the present study.

Table 2 shows highly significant differences in arginase activity between ACRO patients group compared with control group, arginase activity was significantly $p < 0.001$ decreased in patients group $1.95 \pm 0.93$ compared to control $5.98 \pm 2.39$. The serum GH levels of patients with ACRO $5.10 \pm 1.63$ were significantly $p < 0.05$ elevated in comparison to control group $1.02 \pm 0.37$. The serum IGF-1 levels of patients with ACRO $720.12 \pm 310.37$ were significantly $p < 0.05$ elevated in comparison to controls $259.25 \pm 53.94$. The FSG levels in patients with ACRO $98.29 \pm 5.45$ were non-significantly higher $p > 0.05$ compared with control group $87.80 \pm 5.17$. There were no statistically significant $p > 0.05$ differences in serum urea between control group $28.70 \pm 6.32$ and patients with ACRO $26.17 \pm 6.93$, as indicated in Fig. 1, respectively.

Table 2. The serum levels of arginase activity, GH, IGF-1, FSG, and Serum Urea in Patients and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (N = 40)</th>
<th>ACRO (N = 40)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginase U/L</td>
<td>$5.98 \pm 2.39$</td>
<td>$1.95 \pm 0.93$</td>
<td>H.S</td>
</tr>
<tr>
<td>GH ng/mL</td>
<td>$1.02 \pm 0.37$</td>
<td>$5.10 \pm 1.63$</td>
<td>H.S</td>
</tr>
<tr>
<td>IGF-1 ng/mL</td>
<td>$259.25 \pm 53.94$</td>
<td>$720.12 \pm 310.37$</td>
<td>H.S</td>
</tr>
<tr>
<td>FSG mg/dL</td>
<td>$87.80 \pm 5.17$</td>
<td>$98.29 \pm 5.45$</td>
<td>N.S</td>
</tr>
<tr>
<td>Urea mg/dL</td>
<td>$28.70 \pm 6.32$</td>
<td>$26.17 \pm 6.93$</td>
<td>N.S</td>
</tr>
</tbody>
</table>

Abbreviations: ACRO, Acromegaly; GH, Growth Hormone; IGF-1, Insulin Like Growth Factor-1; FSG, Fasting Serum Glucose. $p$-value $>0.05$ is non-significant (N.S), $p$-value $\leq 0.05$ is significant (S), $p$-value $<0.001$ is highly significant (H.S).

As far as we know, this is the first study that dealt with Arg activity in ACRO patients. The current study has found that arginase activity was decreased (this decrease in enzyme activity could be a result of chronic GH signaling in ACRO patients which may inhibit arginase activity), for protecting nitrogen compounds in the body from losing through the urea cycle and enabling the body to carry out its building process using nitrogen-containing proteins. Except for adipose tissue, which has a catabolic effect that results in the breakdown of conserved triglycerides to FFA, GH (in the case of acromegaly GH releases excessively) stimulates an anabolic effect in most tissues.

The current study has found that GH levels in patients group were significantly higher than in...
control group. This elevation was caused by an excess of GH produced by the interior loop of a hormonally active pituitary adenoma. A recent study has reported that GH was higher in ACRO patients compared with controls, which is in agreement with our findings. The current study has found that IGF-1 levels in patients group were significantly higher than in control group. This elevation was caused by excessive GH production from the pituitary glands interior loop, and IGF-1 increased in tandem with GH because IGF-1 production and release are stimulated by GH. As a result, considerable increases in GH levels in the current study are related to significant increases in IGF-1 levels. A recent study has reported that ACRO patients had higher IGF-1 levels than control individuals. A recent study has reported that IGF-1 was higher in ACRO patients compared with controls. The above studies were in agreement with the findings of our study.

The current study has found that there were no FSG significant differences in ACRO group compared with control group. High GH causes improper glucose metabolism, which leads to insulin resistance and, ultimately, diabetes. A recent study has reported that FSG levels in patients were significantly higher compared to controls. This is inconsistent with our current study due to diabetic ACRO patients’ exclusion. In agreement with our findings, a previous study has reported that fasting plasma glucose levels in ACRO patients were not significantly higher compared to control group, because ACRO patients with diabetes mellitus were not included in the study. The current study has found that serum urea levels in patients group were not significantly lower than in control group. A previous study has reported that the effects of GH on protein metabolism include enhanced protein synthesis and reduced breakdown throughout the body and in muscles, as well as reduced amino acid degradation/oxidation and hepatic urea production. A previous study reported that blood urea nitrogen in ACRO patients was reduced, which agrees with our findings.

Table 3 shows highly significant differences in TC levels between control group, and patients with ACRO, TC levels were significantly p<0.001 increased in patients group 175.58±37.22 compared to control group 146.20±12.94. There were highly significant differences in TG levels between control group, and patients with ACRO. TG levels were significantly p<0.001 increased in patients group 152.30±47.49 compared to control group 104.23±22.09. The serum HDL-C levels of patients with ACRO 37.73±4.79 were significantly p<0.05 reduced in comparison to control group 48.06±5.64. There were highly significant differences in LDL-C levels between control group 77.37±11.75, and patients with ACRO 107.39±34.29, LDL-C levels were significantly p<0.0001 increased in patients group compared to control group. There were highly significant differences in VLDL-C levels between control group 20.85±4.40, and patients with ACRO 30.46±9.49. VLDL-C levels were significantly p<0.001 increased in patients group compared with control group, as shown in Fig. 2, respectively.

**Table 3. The levels of lipid profile in patients and control groups.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (N = 40)</th>
<th>ACRO (N = 40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC mg/dL</td>
<td>146.20±12.94</td>
<td>175.58±37.22</td>
<td>H.S</td>
</tr>
<tr>
<td>TG mg/dL</td>
<td>104.23±22.09</td>
<td>152.30±47.49</td>
<td>H.S</td>
</tr>
<tr>
<td>HDL-C mg/dL</td>
<td>48.06±5.64</td>
<td>37.73±7.49</td>
<td>H.S</td>
</tr>
<tr>
<td>LDL-C mg/dL</td>
<td>77.37±11.75</td>
<td>107.39±34.29</td>
<td>H.S</td>
</tr>
<tr>
<td>VLDL-C mg/dL</td>
<td>20.85±4.40</td>
<td>30.46±9.49</td>
<td>H.S</td>
</tr>
</tbody>
</table>

Abbreviations: ACRO, Acromegaly; TC, Total Cholesterol; TG, Triglycerides; HDL-C, High-density lipoprotein-cholesterol; LDL-C, Low-density lipoprotein-cholesterol; VLDL-C, Very low-density lipoprotein-cholesterol. p-value >0.05 is non-significant (N.S), p-value ≤0.05 is significant (S), p-value <0.001 is highly significant (H.S).

**Figure 2. Unpaired t-test for Lipid profile.**
The current study has found that TC levels in patients group were significantly higher than in control group. Elevated TC in ACRO patients is attributed to associated with reduced HDL-C because HDL-C collects excessive cholesterol from the circulatory system and transports it to the liver, where it is broken down and eliminated from the body. Hence, a previous study has reported that TC was higher in ACRO patients in comparison with control group, which is in agreement with our results. The current study has found that TG levels in patients group were significantly higher than in control group. A previous study has interpreted high TG levels in ACRO patients by speculating that the decrease in hepatic triglyceride lipase activity and, possibly, the decrease in lipoprotein lipase activity is at least partially responsible for the development of ACRO’s hypertriglyceridemia. A recent study has found that TG was significantly elevated in ACRO patients compared with control group, which agrees with our findings.

The current study has found that HDL-C levels in patients group were significantly lower than in control group, which matches the results of the recent study. The previous study has observed that HDL abnormalities were linked with reduced Lecithin–cholesterol acyltransferase action and found that ACRO patients’ HDL-C levels were lower than those of control group. The current study showed that LDL-C levels in patients group were significantly higher than in control group. Decreased HDL-C contributes to LDL-C elevation because of the HDL-C participation in reverse cholesterol transport, which transports excess cholesterol from peripheral tissues to the liver. A recent study has found that patients with ACRO had significantly higher LDL-C levels compared to control group, which supports our findings.

The current study has found that VLDL-C levels in patients group were significantly higher than in control group. Because of HDL-C removes excess cholesterol from the bloodstream, high VLDL-C levels are associated with reduced HDL-C levels. A recent study has found that VLDL levels in patients group were significantly higher compared to control group, which agrees with our results. Table 4 shows the correlation coefficient values of arginase with other parameters in ACRO patients.

### Table 4. Correlation of arginase with other parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pearson Correlation</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Age</td>
<td>0.212</td>
<td>0.188</td>
</tr>
<tr>
<td>BMI</td>
<td>0.043</td>
<td>0.790</td>
</tr>
<tr>
<td>GH</td>
<td>-0.066</td>
<td>0.688</td>
</tr>
<tr>
<td>IGF-1</td>
<td>0.092</td>
<td>0.574</td>
</tr>
<tr>
<td>FSG</td>
<td>0.274</td>
<td>0.087</td>
</tr>
<tr>
<td>TC</td>
<td>0.072</td>
<td>0.657</td>
</tr>
<tr>
<td>TG</td>
<td>-0.048</td>
<td>0.767</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.018</td>
<td>0.912</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.088</td>
<td>0.589</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>-0.048</td>
<td>0.767</td>
</tr>
<tr>
<td>Urea</td>
<td>0.159</td>
<td>0.326</td>
</tr>
</tbody>
</table>

### Conclusion

Patients with ACRO have lower levels of serum arginase activity and non-significantly lower levels of serum urea, which means their bodies retain more nitrogen compounds for use in building processes. Also, they have an increased risk factor of developing cardiovascular problems because of their undesirable lipid profile (elevated LDL-C and reduced HDL-C levels in addition to higher levels of TG, TC, and VLDL-C).

### Acknowledgment

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### Author’s Declaration

- **Conflicts of Interest:** None.
- **We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.**
- **Authors sign on ethical consideration’s approval.**
Author’s Contribution Statement

F.K.M. performed the statistical analysis, performed the experiments with the help of S.A.A. and B.A.A., and wrote the manuscript in consultation with S.A.A.

and B.A.A., S.A.A. designed the research, and B.A.A. collected samples.

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تقييم نشاط إنزيم الأرجنيز وبعض المتغيرات الكيموحيوية في أمصال المرضى المصابين بتضخم الأطراف

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الخلاصة

هدفت هذه الدراسة إلى دراسة تأثير زيادة هرمون النمو على فعالية إنزيم الأرجنيز في أمصال مرضى تضخم الأطراف العراقيين. تم قياس بعض المتغيرات الكيموحيوية مثل هرمون النمو (GH)، عامل النمو شبيه الأنسولين (IGF-1)، البروتين الدهني عالي الكثافة (HDL-C)، البروتين الدهني منخفض الكثافة (TC)، البروتين الدهني منخفض الكثافة (TG)، البروتين الدهني منخفض الكثافة (LDL-C)، والبروتين الدهني منخفض الكثافة (VLDL-C). شارك في هذه الدراسة ثمانون شخصاً تتراوح أعمارهم بين 25 و 65 عاماً، كان 40 منهم من مرضى تضخم الأطراف العراقيين والـ 40 الباقين كانوا أصحاء. تم طباعة جميع المشاركين من حيث العمر والجنس وتم حساب مؤشر كتلة الجسم لكل مجموعة. انخفضت فعالية إنزيم الأرجنيز في مرضى ACRO بشكل ملحوظ بالمقارنة بالجهاز الضامن من حيث GH و IGF-1 بشكل ملحوظ، بينما ارتفعت مستويات HDL-C بشكل ملحوظ، بينما ارتفعت مستويات LDL-C بشكل ملحوظ، بينما ارتفعت مستويات VLDL-C بشكل ملحوظ، بينما ارتفعت مستويات FSG في مرضى ACRO بشكل ملحوظ، بينما ارتفعت مستويات البلازما من حيث TC بشكل ملحوظ، بينما ارتفعت مستويات البلازما من حيث TG بشكل ملحوظ، بينما ارتفعت مستويات البلازما من حيث IGF-1 بشكل ملحوظ، بينما ارتفعت مستويات البلازما من حيث GH بشكل ملحوظ، بينما ارتفعت مستويات البلازما من حيث LDL-C بشكل ملحوظ، بينما ارتفعت مستويات البلازما من حيث VLDL-C بشكل ملحوظ. هذه النتائج تتطلب تحسينات في تكنولوجيا التشخيص وعلاج مرضى ACRO. الاستخدام في تحسينات في شروط البيئة، والعلاجات، والرعاية الصحية، والتعليم.

الكلمات المفتاحية: إنزيم الأرجنيز، تضخم الأطراف، هرمون النمو، عامل النمو شبيه الأنسولين، صورة الدهون.