

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

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Received 21/03/2023, Revised 02/10/2023, Accepted 04/10/2023, Published Online First 20/05/2024,
Published 01/12/2024



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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^{1,2}. 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^{3,4}. This pathology occurs on average 40 to 70 people per

million⁵. The ACRO has a significant diagnostic delay that is linked to a higher risk of complications and deteriorating psychosocial conditions⁶. The GH is essential for normal postnatal growth⁷, 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^{8,9}. The GH stimulates the formation of bones and modulates the metabolism of fats, carbohydrates, nitrogen, and minerals as well as electrolyte balance¹⁰. 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-1¹¹. 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 bloodstream¹².

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 processes¹³. 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 insulin¹⁴.

L-arginase (Arg) (EC 3.5.3.1) catalyzes, the last step in the urea cycle, is a binuclear manganese

metalloenzyme¹⁵, 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 arginase¹⁶. 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 activity¹⁷. 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 activity¹⁸. 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 diabetes¹⁹. Currently, Arginase inhibitors are regarded as promising therapies for the treatment of a variety of pathologies²⁰. 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 Porembaska's method, arginase activity in serum was examined manually²¹. 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 \pm 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±10.30 years and patients with ACRO 46.35±9.25 years. The body mass index (BMI) value was found to be highly significant $p < 0.001$ in patients with ACRO 33.41±3.64 kg/m² compared to control group 24.31±2.05 kg/m².

Table 1. The study subjects' characteristics.

Parameters	Control (N = 40)	ACRO (N = 40)	p-value
Age year	44.69±10.30	46.35±9.25	N.S
BMI kg/m ²	24.31±2.05	33.41±3.64	H.S

Abbreviations: ACRO, Acromegaly; BMI, Body Mass Index. 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).

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 study²².

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±0.93 compared to control 5.98±2.39. The serum GH levels of patients with ACRO 5.10±1.63 were significantly $p < 0.05$ elevated in comparison to

control group 1.02±0.37. The serum IGF-1 levels of patients with ACRO 720.12±310.37 were significantly $p < 0.05$ elevated in comparison to controls 259.25±53.94. The FSG levels in patients with ACRO 98.29±5.45 were non-significantly higher $p > 0.05$ compared with control group 87.80±5.17. There were no statistically significant $P > 0.05$ differences in serum urea between control group 28.70±6.32 and patients with ACRO 26.17±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.

Parameters	Control (N = 40)	ACRO (N = 40)	p-value
Arginase U/L	5.98±2.39	1.95±0.93	H.S
GH ng/mL	1.02±0.37	5.10±1.63	H.S
IGF-1 ng/mL	259.25±53.94	720.12±310.37	H.S
FSG mg/dL	87.80±5.17	98.29±5.45	N.S
Urea mg/dL	28.70±6.32	26.17±6.93	N.S

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 ≤ 0.05 is significant (S), p -value < 0.001 is highly significant (H.S).

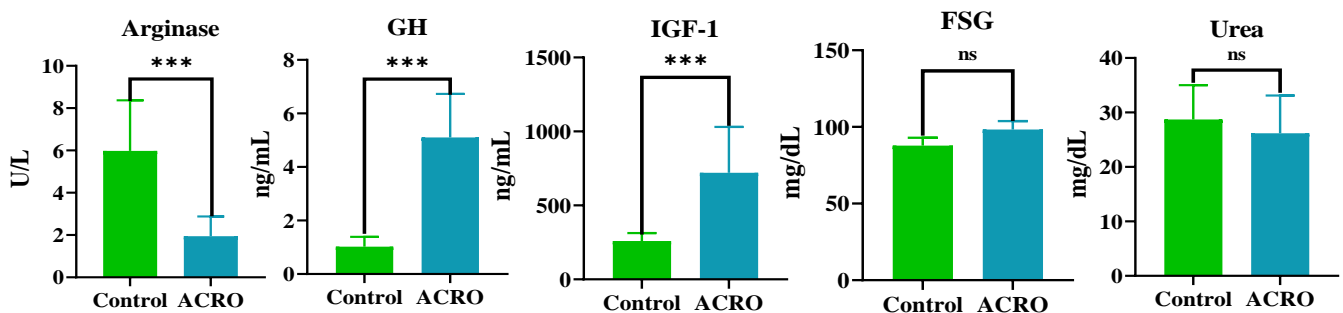


Figure 1. Unpaired t-test for Arginase Activity, GH, IGF-1, FSG, and Serum Urea.

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 tissues²³.

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 anterior lobe 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 findings²⁴.

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 anterior 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-I levels than control individuals²². A recent study has reported that IGF-1 was higher in ACRO patients compared with controls²⁴. 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^{25,26}. 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 study²⁷.

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 production²⁸. A previous study reported that blood urea nitrogen in ACRO patients was reduced, which agrees with our findings²⁹.

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 ± 7.49 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.

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

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).

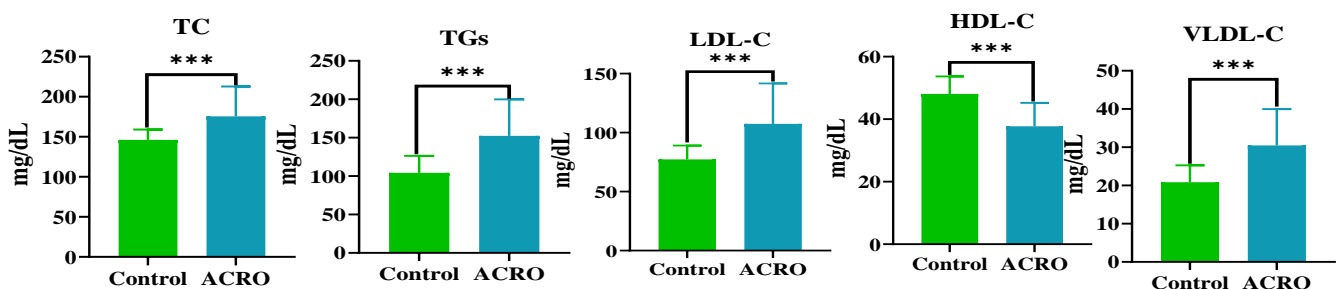


Figure 2. Unpaired t-test for Lipid profile.

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 body³⁰. Hence, a previous study has reported that TC was higher in ACRO patients in comparison with control group, which is in agreement with our results²⁵.

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 hypertriglyceridemia³¹. 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 study²⁵. 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 group³².

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 liver³⁰. A recent study has found that patients

with ACRO had significantly higher LDL-C levels compared to control group, which supports our findings²⁵.

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 levels³⁰. A recent study has found that VLDL levels in patients group were significantly higher compared to control group, which agrees with our results²⁵. Table 4 shows the correlation coefficient values of arginase with other parameters in ACRO patients.

Table 4. Correlation of arginase with other parameters.

Parameters	Arginase	
	Pearson Correlation	p-value
Age	0.212	0.188
BMI	0.043	0.790
GH	-0.066	0.688
IGF-1	0.092	0.574
FSG	0.274	0.087
TC	0.072	0.657
TG	-0.048	0.767
HDL-C	0.018	0.912
LDL-C	0.088	0.589
VLDL-C	-0.048	0.767
Urea	0.159	0.326

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

The authors like to express their gratitude to Mustansiriyah University, College of Science,

Chemistry Department, and National Diabetes Center in Baghdad, Iraq.

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.
- Ethical Clearance: The project was approved by the local ethical committee at Mustansiriyah University.
- No human studies are present in the manuscript.

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 انزيم الارجنيز وبعض المتغيرات الكيموحيوية في امصال المرضى المصابين بتضخم الأطراف

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الخلاصة

هدفت هذه الدراسة الى دراسة تأثير زيادة هرمون النمو على فعالية انزيم الارجنيز في امصال مرضى تضخم الاطراف العراقيين. تم قياس بعض المتغيرات الكيموحيوية مثل هرمون النمو (GH)، عامل النمو شبيه الانسولين (IGF-1)، فحص السكر الصيامي (FSG)، اليوريا، الكوليسترول (TC)، الدهون الثلاثية (TG)، البروتين الدهني عالي الكثافة (HDL-C)، البروتين الدهني منخفض الكثافة (LDL-C)، والبروتين الدهني منخفض الكثافة جدا (VLDL-C). شارك في هذه الدراسة ثمانون شخصاً تتراوح أعمارهم بين 25 و 65 عامًا، كان 40 منهم من مرضى تضخم الاطراف العراقيين والـ 40 الباقين كانوا أصحاء. تمت مطابقة جميع المشاركين من حيث العمر والجنس وتم حساب مؤشر كتلة الجسم لكل مجموعة. انخفضت فعالية انزيم الارجنيز في مرضى ACRO بشكل ملحوظ $p < 0.05$ مقارنة بالمجموعة الضابطة، وقد انخفض كذلك HDL-C بشكل ملحوظ، بينما ارتفعت مستويات GH و IGF-1 و TC و TG و LDL-C و VLDL-C بشكل ملحوظ $p < 0.05$. ولم تكن هناك فروق ذات دلالة إحصائية $P > 0.05$ في مستويات FSG و اليوريا بين المرضى والأصحاء. إن نتائج الدراسة الحالية أوجت إلى أن مرضى ACRO لديهم مستويات أقل من فعالية انزيم الارجنيز المصل (بشكل ملحوظ) ومستويات أقل من مصل اليوريا (بشكل غير ملحوظ)، مما يعني أن أجسامهم تحتفظ بمزيد من مركبات النيتروجين لاستخدامها في عمليات البناء.

الكلمات المفتاحية: انزيم الارجنيز، تضخم الاطراف، هرمون النمو، عامل النمو شبيه الانسولين، صورة الدهون.