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The Study of Prolidase Level and Some Biochemical Variables in Iraqi Patients with Acromegaly

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Abstract

Acromegaly is a condition that results from excessive growth hormone production by the anterior pituitary gland. Prolidase (PLD) is the only known human enzyme that can hydrolyze dipeptides with an amino acid at their C terminus. Reports indicate that PLD activity serves as a marker for oxidative stress in numerous disorders such as diabetes, diabetic neuropathy, chronic liver diseases, and osteoporosis. The current study aims to estimate the values of PLD, its correlation with the rest of the parameters, and ROC in acromegaly patients. A group of 61 patients with confirmed acromegaly were collected from the National Diabetic Center, Mustansyrriah University, and 60 control groups were analyzed in the same place for the parameters of the biochemical study. Acromegalic patients had changes in the levels of biochemical markers like PLD, growth hormone, insulin-like growth factor-1, and fasting blood glucose. There was a high difference ($p < 0.001$) between the patient group and the healthy control group. The current study shows that PLD levels are higher in patients with acromegaly than in the healthy control group. The sensitivity and specificity for PLD were high in the patient group versus the healthy control. The PLD may be a novel biomarker of acromegaly activity, and there may be an increase in fasting blood glucose, growth hormone, and IGF-1 in acromegaly patients.

Keywords: Acromegaly, growth hormone, hyperglycemia, insulin like growth factor-1, prolidase.

1. Introduction

Acromegaly is an uncommon type of chronic illness marked by overproduction of the hormones growth hormone (GH) and insulin-like growth factor 1 (IGF-1). Pituitary adenomas are the root cause of more than 95% of instances of acromegaly [1-3]. Acromegaly often develops slowly and subtly [4]. Its prevalence is 130 cases per million, although its annual incidence is between 4 and 6 cases per million per year [5]. Acromegaly is linked to a variety of systemic problems, including hypopituitarism, osteoarthritis, metabolic issues like respiratory problems, perhaps increased risks of certain neoplasias, vertebral fractures, insulin resistance, hyperglycemia, and hyperlipidemia, as well as a poor quality of life. Vertebral fractures and a

lower quality of life are two of these more recent effects. For many years, cardiovascular disease was thought to be the primary cause of mortality [6-8]. Surgical, pharmaceutical, and radiotherapeutic methods treat acromegaly. Multimodal therapy can usually control acromegaly by stopping GH hypersecretion, lowering IGF-I levels, and stopping tumor growth. This can also reduce the symptoms and other health problems that come with it [9,10]. The anterior pituitary secretes the peptide hormone known as human GH. Its main function is the regulation of postnatal growth and metabolism, and it affects many different human tissues in a pleiotropic manner [11]. The somatotroph cells in the proximal portion of the pituitary gland produce and release growth hormone. The hypothalamus and GH action mediators control GH secretion. The GH-releasing hormone (GHRH), GH-releasing peptide (ghrelin), IGF-1, and somatostatin are some examples of regulatory substances. GH/IGF1 system disorders are mostly caused by either too much GH (acromegaly or gigantism) or not enough GH [12]. Insulin-like growth factor 1 (IGF-1), which can directly decrease growth hormone release from the somatotrophins or indirectly through an increase in somatostatin, also impacts hormone release. It also reduces the release of GHRH, which in turn inhibits the creation of GH [13]. Growth hormone (GH) and IGF1 control the two most prevalent proteins in musculoskeletal tissues, collagen and myofibrillar protein [14]. Prolidase (PLD) is a critical enzyme that helps provide the proline needed for collagen production and cell development. The PLD absence causes clinical signs of collagen deficiency, while increased PLD activity is associated with fibrosis. Therefore, scientists believe that PLD constrains the control of collagen synthesis [15]. The PLD is the sole known human enzyme capable of hydrolyzing dipeptides with an amino acid at their C terminus. This causes the production of free proline, which the cell may then utilize. In collagen metabolism, this action is very crucial [16]. The PLD is present in the brain, heart tissue, uterine tissue cells, spleen, mononuclear cells, neutrophils, red blood cells, gut, and red blood cells. It plays a significant part in several physiological processes. Furthermore, it contributes significantly to pathological conditions like injury and infection recovery, vascular growth that fosters cancer, and the dissemination of cancer [17]. The PLD deficiency (PD) is characterized by a variety of clinical signs, the most common of which are persistent respiratory infections, mental impairment, and chronic skin ulcerations [16]. The PLD activity frequently triggers this condition. To date, researchers have identified only a little over one hundred people with PD, a rare recessive genetic condition. The PLD gene has 35 variants in PD patients, including 9 insertions and deletions and 16 missense mutations [18]. Furthermore, various clinical states have observed changes in PLD enzyme activity, making it a valuable biochemical marker for evaluating the severity of the disease [19]. The study's goal is to estimate the values of PLD, correlation, and Roc in acromegaly patients.

2. Materials and Methods

2.1 Patients selection

Sixty-one acromegalic patients were enrolled in the study, plus sixty non-acromegalic patients as the control group. They were studied during the period from November 2022 to February 2023. They attend the National Diabetic Center at Mustansyrriah University monthly for follow-up and to receive their monthly injection of long-acting repeatable octreotide (Sandostatin-LAR) after ethical consent obtained from the review board and verbal consent of participation from the subjects.

2.2 Inclusion and exclusion criteria

The inclusion criteria included patients with acromegaly aged 30-65 years old, and the exclusion criteria are thyroid disease, heart disease, and kidney disease, as well as pregnant women. The study measured the following parameters: PLD, GH, and IGF-1.

2.3 Biochemical assay

The PLD level was measured from a serum sample by the (enzyme-linked immunosorbent assay (ELISA)) using a kit (My BioSource, USA), while GH and IGF-1 were measured using the sandwich chemiluminescence immunoassay technique (ELISA) Blood samples (5 mL) were collected from acromegalic patients and healthy subjects, then centrifuged at 3000 rpm for 10 min., and kept at a temperature of -20˚C.

2.4 Statistical analysis

The significance of the results was evaluated using SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). Summary data are presented as means ± SD. The statistical differences between continuous variables were analyzed using an independent sample Student's t-test. Other tests have been used. A graphical plot known as the Receiver Operating Characteristic (ROC) illustrates the diagnostic ability of a binary classifier system as its discrimination threshold varies.

3. Results

Table 1 shows the baseline characteristics of the patients and their healthy control counterparts. The patient group has a higher level of PLD than the healthy control group. The PLD is 694.57±122.12 in the patient group and 411.64±62.86 in the control group ($p < 0.001$). Fasting blood glucose (FBG) is 143.84±83.02 in acromegalic patients and 93.58±11.56 in healthy controls; the difference is highly significant. The GH in acromegalic patients is 7.05±3.53, whereas in the control it is 0.70±0.29, and the difference is highly significant ($p < 0.001$). Moreover, IGF-1 is 482.96±238.28 in patients and 105.28±6.5 in healthy controls; the difference is highly significant.

Table 1. The baseline characteristics of the acromegalic patients and healthy control group**.**

Parameters	Acromegaly	Control	*P* value
Number	61	60	-
Male/Female	35/26	30/30	-
Age (Years)	49.57±10.38	46.98±5.64	> 0.05
PLD (U/L)	694.57±122.12	411.64±62.86	< 0.001
FBG (mg/dL)	143.84±83.02	93.58±11.56	< 0.001
GH (ng/ml)	7.05±3.53	0.70±0.29	< 0.001
IGF-1 (ng/ml)	482.96±238.28	105.28±6.5	< 0.001

Table 2 shows the biochemical variables (PLD, FBG, G-H, and IGF-1) in patients and the control group according to their gender. In the patient groups, the difference between males and females did not reach statistical significance, as evidenced by the *P*-values of 0.378, 0.839, 0.188, and 0.301 for GH, IGF-1, FBG, and PLD, respectively. However, in the healthy controls, there was a highly significant difference in growth hormone between male and females.

Table 2. The biochemical variables in patients and control group according to their gender.

Parameters	Acromegaly			Healthy Control		
	Male	Female	*P* value	Male	Female	*P* value
PLD (U/L)	680.51±118.21	714.49±127.30	0.301	414.81±63.28	408.24±63.38	0.695
FBG (mg/dL)	131.71±75.94	160.15±90.65	0.188	93.13±10.65	94.03±12.57	0.766
GH (ng/mL)	7.40±3.65	6.59±3.38	0.378	0.62±0.19	0.79±0.34	0.019
IGF-1(ng/mL)	477.54±206.98	490.26±279.11	0.839	105.67±6.16	104.90±6.99	0.654

Table 3 shows the correlation of the studied molecules with age, PLD, GH, and IGF-1. The relationship is negative with age for PLD, FBG, GH, and IGF-1. We find a statistically significant correlation between age and the following variables: GH and IGF-1 (*P*-values of 0.005 and 0.001, respectively).

The PLD has a negative correlation only with age, but its correlation with the other variables is positive; however, those who reach statistical significance are with GH (*P*-value 0.037). Fasting blood glucose correlates negatively only with age and positively with other markers; however, the relation reaches statistical significance with GH only (*P*-value is 0.001). Growth hormone correlates negatively with age and FBG, while its correlation with the others is positive; however, the correlation reaches statistical significance between IGF-1 and PLD (*P*-values of 0.001 and 0.037, respectively). Insulin, like growth factor-1, correlates negatively with age, but positively with FBG, GH, and PLD. These correlations between IGF-1 and age and GH reach statistical significance (*P*-values of 0.001 and 0.001, respectively).

Table 3. The correlations between the parameters in acromegalic patients.

Correlations						
		Age	**FBG**	**GH**	**IGF-1**	**PLD**
Age	Pearson Correlation	1	-0.174	-0.360**	-0.398**	-0.045
	Sig. (2-tailed)		0.181	0.005	0.001	0.735
	N	61	61	61	61	61
PLD	Pearson Correlation	-0.045	0.075	0.279*	0.162	1
	Sig. (2-tailed)	0.735	0.575	0.037	0.225	
	N	61	61	61	61	61
FBG	Pearson Correlation	-0.174	1	0.467**	0.174	0.075
	Sig. (2-tailed)	.181		0.001	0.180	0.575
	N	61	61	61	61	61
GH	Pearson Correlation	-0.071	-0.011	1	0.351**	0.279*
	Sig. (2-tailed)	0.585	0.930		0.006	0.037
	N	61	61	61	61	61
IGF-1	Pearson Correlation	-0.398**	0.174	0.525**	1	0.162
	Sig. (2-tailed)	0.001	0.180	0.001		0.225
	N	61	61	61	61	61

The ROC curve indicates a PLD area under the curve (AUC) of 0.976, a 95% confidence interval (CI) with sensitivity and specificity of 93.4 and 91.7, respectively, with a *p*-value of less than 0.001, and a best cut-off point of 528.598 pg/mL. That means the test value less than (528.598 pg/mL) is considered healthy, whereas the value greater than (528.598 pg/mL) represents the unusual case, as shown in **Figure 1**.

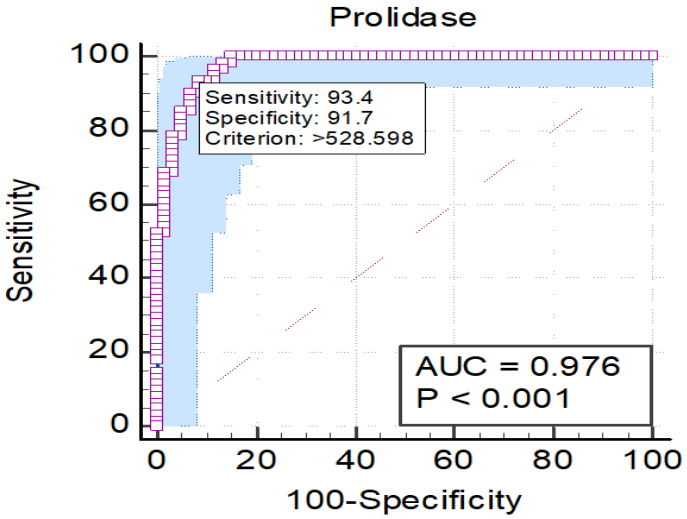

Figure 1. The ROC curve of PLD.

4. Discussion

This study conducted on sixty-one patients with acromegaly and sixty with non-acromegaly. To our knowledge, only one study has reported higher levels of PLD in acromegaly. The results are in agreement with the Tabur *et al*., study [15]. Patients with acromegaly and GH insufficiency frequently experience morbidity due to the disproportionate expansion of their muscles and bones [20, 21], and symptoms affecting bone, joints, muscles, tendons, and joints have a significant influence on both patient groups' quality of life [22].

The GH/IGF-1 axis also significantly influences collagen modulation [23, 24]. The acromegaly group had considerably greater PLD activity than the healthy control group, according to the current study. The higher PLD activity suggests that collagen management remains unattainable, even when the condition is under control [25].

Growth hormone and IGF-1 regulate collagen and myofibrillar protein, the two most prevalent proteins in joint tissues. The coarsened facial features and acral overgrowth of the hands and feet in acromegaly patients suggest a correlation between GH/IGF1 and collagen production. Moreover, collagen synthesis appears to be susceptible to hormone-regulatory treatment as well as circulating GH and IGF1 levels [22]. The onset of osteoarthritis has been associated with PLD activity and oxidative stress [26, 27].

There are few studies examining the levels of oxidative stress in individuals with acromegaly. According to Nishizawa *et al*., individuals with acromegaly have higher levels of reactive stress [28]. A recent study by Anagnostis et al. compared 15 people with acromegaly to controls of the same age and gender and found that the acromegaly patients had higher levels of oxidative stress and lower antioxidant capacity [29]. Additionally, joint disease in acromegaly patients persists even after the condition's pharmacologic management [30, 31].

As a result, PLD may play a part in the development of this degenerative joint disease in people who are acromegalic, since it is a key biomarker for controlling this process.

5. Conclusion

The current study shows that PLD levels are higher in patients with acromegaly than in the healthy control group. The sensitivity and specificity for PLD were high in the patient group

versus the healthy control. The PLD may be a novel biomarker of acromegaly activity, and there may be an increase in fasting blood glucose, growth hormone, and IGF-1 in acromegalic patients.

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Conflict of Interest

The authors declare there is no conflict of interest.

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None.

Ethical Clearance

This study was approved by the scientific committee in the College of Science for Women, and a verbal consent from was obtained from each participant enrolled in the study.

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