







## Insulin Like Growth Factor Binding Protein 7 as a Novel Diagnostic Marker in Sera of Iraqi Patients with Acromegaly

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### Abstract

The purpose of this study was to measure serum levels of insulin-like growth factor-binding protein (IGFBP7), Insulin-like Growth Factor 1 (IGF-1), Growth Hormone (GH), Interleukin 6 (IL-6) and insulin in acromegaly patients and healthy controls. The acromegaly group had 60 patients, while the population group had 30 people who had never had acromegaly before. The concentration of IGFBP7, IGF-1, GH, IL-6, and insulin were determined. The results of the present study indicate that IGFBP7 level in the acromegaly group was significantly lower (1.690.07 ng/mL vs. 2.740.12 ng/mL, respectively,  $p = 0.001$ ). IGF-1, GH, IL-6, and insulin concentrations were also significantly higher in acromegaly patients. The diagnostic accuracy (2.194) was excellent in separating acromegaly from the healthy patient group, and the area under the curve (AUC = 0.871,  $p = 0.001$ ). From the results of the present study, we can conclude that serum IGFBP7 can be used to distinguish acromegaly patients from healthy individuals.

**Keywords:** Acromegaly, Growth hormone, Insulin, Insulin-like Growth Factor 1, Insulin like growth factor binding protein 7.

### Introduction

Acromegaly is characterized by excessive growth hormone (GH) release, which is usually induced by a pituitary adenoma, as well as an excess of Insulin like growth factor1(IGF-1) <sup>1</sup>. Circulating IGF-1 reduces GH secretion both directly and indirectly by promoting hypothalamic somatostatin secretion in the pituitary. IGF-1 has a variety of systemic, autocrine, and paracrine actions, each of which is unique to the tissues, cellular pathways, and metabolic conditions in which it occurs. Furthermore, certain downstream parts of the GH and IGF-1/insulin signaling pathways are shared, facilitating crosstalk between

these pathways. Interactions with other hormones and growth factors influence IGF-1's effects<sup>2</sup>. As a result, as observed decades ago, uncontrolled patients with acromegaly typically develop diabetes or at least impaired glucose tolerance (IGT)<sup>3</sup>.

Invertebrates and vertebrates alike need the IGF, which is an evolutionarily conserved polypeptide, for cell growth, proliferation, differentiation, reproduction, and aging <sup>4</sup>. Development, which begins with pregnancy and lasts throughout life, depends on the (IGF) signaling axis <sup>5</sup>. The IGF system is composed of IGF-I, IGF-II, IGF receptors (IGF-IR), and IGF binding

proteins (IGFBPs). The IGFBPs have a role in regulating and modulating the biological actions of IGF<sup>4,5</sup>. The IGFBPs can affect IGF's distribution in tissues, binding to cell receptors, and blood circulation half-life. They can be found in blood circulation, intracellular tissues, and extracellular tissue fluid. Since there are seven IGFBPs, IGF's biological usage and signal transduction are intricate<sup>6</sup>. In animal serum and extracellular fluids, IGFBPs have the power to keep IGFs from deteriorating and control any potential biological roles they may have<sup>7</sup>.

The IGFBP7 was the first part of the proteins associated to IGFBP that was discovered. It is sometimes referred to as insulin-like growth factor-related protein-1 (IGFBP-rp1)<sup>5</sup>. It is a secreted protein called (IGFBP7) that binds insulin, vascular endothelial growth factor A, and insulin-like growth factor 1 (IGF-1)<sup>8,9</sup>. Many different species have been used to clone the IGFBP7. The IGFBP7 molecule has an IGFBP motif (GCGCCXXC) at the N-terminus in a domain with

12 conserved amino acids (cysteines). IGFBP7's C-terminus is different from that of other IGFBPs since it only contains one cysteine and lacks the conserved cysteines<sup>4</sup>. Insulin resistance is caused by the IGFBP7 protein's high affinity for insulin, preventing insulin from binding to its receptor and blocking insulin-stimulated autophosphorylation of insulin receptor subunits and phosphorylation of insulin receptor substrate I(IRS-1)<sup>10,11</sup>. The majority of healthy organs, including the liver, brain, pancreas, and skeletal muscle, release IGFBP7 into the bloodstream, where it is produced at both the protein and mRNA levels. Although insulin, IGF-1, and IGF-2 can all connect to insulin receptors, IGF-2R can only bind to IGF-2<sup>4,12</sup>.

The aim of this study was to determine the diagnostic accuracy of serum IGFBP7 in acromegaly, and compare some related markers including fasting blood glucose (FBG), IGF-1, GH, IL-6, Insulin, and HOMA-IR between acromegaly patients and healthy group.

## Materials and Methods

### Patients

From November 2021 to January 2022, 60 male acromegaly patients were enrolled at the National Diabetes / Mustansiriyah University in Baghdad, Iraq, and were compared to 30 healthy individuals as control group (age and sex matched). Patients diagnosed with acromegaly and treated on monthly basis, ranging in age from 25 to 60. Before participating in this trial, all patients gave their consent, which was signed and dated.

### Methods

Ten milliliters of venous blood were collected using a disposable plastic syringe with a (10 ml) capacity. It was placed in a basic disposable gel tube to clot. The blood was then centrifuged for 10 minutes at 3000 rpm to obtain serum. A thorough history was taken of each patient. All of

the patients were subjected to a physical and medical assessment (height, weight). FBG was measured using Tool Set (Biolabo). The DRG-USA provided an ELISA kit for measuring serum insulin. GH, IL-6, IGFP-7, and IGF-1 serum were tested using an ELISA kit provided by Abcam, the United Kingdom. The calculation the homeostasis model assessment (HOMA) was determined fasting insulin and glucose.<sup>13</sup>

### Statistical Analysis

SPSS version 24 was used for statistical analysis and reporting of the data collected. An unpaired t-test was used to compare the significance of differences between the two groups, with p significant. The person correlation test, also known as the receiver operating characteristic ROC, has been used to identify between variables.

## Results and Discussion

The average age and standard error distribution of acromegaly patients ( $47.27 \pm 1.48$ ) year and healthy people ( $49.80 \pm 1.06$ ) year is shown in Table 1, with

$P \leq 0.005$  for both groups. High significant difference increase in FBG, IGF-1, GH, IL-6, and Insuline, HOMA-IR in acromegaly patients group ( $p < 0.001$ )

when compared with healthy subjects group as shown in Table 1.

**Table 1. The mean  $\pm$  standard error of Age, FBG , IGF-1, GH, IL-6, Insulin and , HOMA-IR in acromegaly patients and control groups.**

Parameters	Patients group	Control	P value
Age (year)	47.27 $\pm$ 1.48	49.80 $\pm$ 1.06	N.S
FBG (mg/dl)	117.9 $\pm$ 3.54	90.8 $\pm$ 1.90	<0.001
IGF-1(ng/ml)	538.33 $\pm$ 41.96	314.4 $\pm$ 6.89	<0.001
GH(ng/ml)	15.29 $\pm$ 1.12	1.50 $\pm$ 0.03	<0.001
IL-6(pg/ml)	15.24 $\pm$ 1.62	8.16 $\pm$ 0.75	<0.001
Insulin (ng/dl)	18.9 $\pm$ 1.69	3.62 $\pm$ 0.302	<0.001
HOMA-IR	8.92 $\pm$ 2.91	4.02 $\pm$ 1.87	<0.001

The data was evaluated using either the mean (mean), and the standard error ( $\pm$  SE ).

**Table 2. The serum levels of IGFBP7 in acromegaly patients and control.**

Parameters	Patients group	Control	P value
IGFBP7 (ng/ml)	1.69 $\pm$ 0.07	2.74 $\pm$ 0.12	<0.001

The data was evaluated using either the mean (mean) , and the standard error ( $\pm$  SE ).

The IGFBP7 levels in the acromegaly patients group were highly significantly different in patients (1.69 $\pm$ 0.07)ng/ml, with (p<0.001) when compared with the control group(2.74 $\pm$ 0.12)ng/ml, respectively as shown as in Table 2 .

#### The Correlation Study between IGFBP7 and Other Parameters

The IGFBP7 with other markers (IGF-1, IL-6, HOMA-IR and Insulin) in acromegaly patients and control were studied in a correlation research.

In acromegaly patients group there was a significant associations between serum IGFBP7 and other parameters including IL-6, Insulin and HOMA-IR and no significant associations between serum IGFBP7 with FBG. While in control group there was a significant associations between serum IGFBP7 and FBG, Insulin and HOMA-IR and no significant associations between serum IGFBP7 with IL-6 as shown in Table 3.

**Table 3. Correlation between IGFBP7 with FBG, IL-6, HOMA-IR and Insulin in acromegaly patients and control.**

Parameters	Patients group R value(P value)	Control group R value(P value)
FBG	-0.045(0.733)	0.370 (0.04) *
IL-6	0.352 (0.00)*-	0.099(0.604)
Insulin	- 0.284(0.020*)	- 0.401(0.028)*
HOMA-IR	- 0.391(0.031*)	- 0.521(0.039*)

Previous studies indicated that IGFBP7 was found as a new biomarker for a variety of disorders, including heart failure, cardiomegaly, etc. The IGFBP7's function in the development and

evolution of diabetes, acute kidney injury (AKI), obesity, chronic obstructive pulmonary disease (COPD), malignancies, and other diseases has been investigated in a number of research<sup>14</sup>.

There has never been any research that has defined the role of IGFBP7 in acromegaly development. In the sera of Iraqi patients, we discovered that IGFBP7 appears to be a good prognostic marker, which is the first study to address this topic to our knowledge.

The IGFBP7 levels in serum were significantly lower in patients with acromegaly compared to healthy individuals or control group. The results of our current study indicate that this protein is involved in the development of acromegaly. Numerous studies have shown that the binding proteins cause malignant cells to develop less quickly and undergo more apoptosis<sup>15</sup>. IGFBP7 can control the growth-suppressing effects of the TGF superfamily, and TGF-1 and RA treatment of cells can also boost IGFBP7 expression. In earlier studies, it was discovered that the IGFBP7 inhibits the growth of tumors in the bladder, liver, uterine cervix, esophagus, lung, thyroid, neck, and head. The expression of this potentially significant chemical has been linked to the differentiation of cancer cells<sup>4,16</sup>.

This protein interacts with the insulin receptor (InsR) to bind insulin with a high affinity (500 times greater than other IGFBPs), lowering blood levels of free insulin, blocking insulin binding to the InsR, and reducing the physiological response to insulin<sup>17</sup>. IGFBP7 concentration measurements reflect genuine insulin production by the pancreas

<sup>14</sup>. IGF-I is a hormone that is generated mostly by the liver in response to growth hormone stimulation and has a structure similar to insulin. Because insulin and IGF-I receptors are related, they share a number of signaling pathways as well as cell and biological responses<sup>18</sup>. As a result, it's difficult to tell the difference between the functions of insulin and IGF-1 in the development of diabetes and the advancement of IR<sup>19</sup>. IGF-1 and the immune system have complicated relationships.

Through several forms of intracellular interaction, pro-inflammatory cytokines appear to decrease the effects of IGF-1. IGF-1 receptor activated pathways and pro-inflammatory signaling pathways, for example, sharing the signaling components mitogen-activated protein kinase (MAPK), especially extracellular signal regulated protein kinases (ERK) 1/2<sup>20,21</sup>.

#### ROC Curve Analysis

The effectiveness of blood IGFBP-7 levels to distinguish acromegaly patients from healthy persons was investigated using ROC curve analysis. (Table 5; Fig. 1). Acromegaly had a much larger ROC curve than the diagnostic test, indicating greater validity (high sensitivity 80 and specificity 86.7). The area under the curve of the ROC curve for the presence of an acromegaly diagnosis was 0.871 (p 0.001), indicating that the ideal level of acromegaly correct prediction was achieved

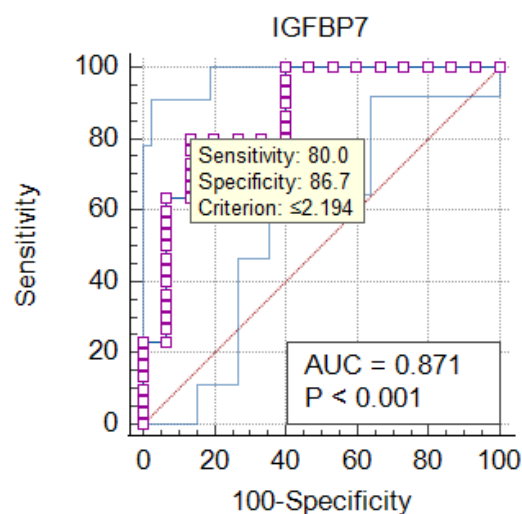


Figure 1. The Roc curve for IGFBP-7 concentration in acromegaly and normal healthy groups

**Table 5. IGFBP-7's validity and AUC in separating patients with acromegaly from the healthy group.**

Variable	AUC	P-Value	Optimum cut off value	Sensitivity	Specificity	Accuracy	PPV	NPV
IGFBP-7	0.871	0.001	2.194	80.7	86	0.6667	92.3	68.4

AUC is an abbreviation for the area under the curve is NPV stands for negative predictive value, while PPV stands for positive predictive value.

## Conclusion

The significant reduction in the level of IGFBP7 and significant elevation in the levels of (FBG, GH, IGF-1, insulin, HOMA-IR, IL-6) in patients with acromegaly. Acromegaly cause insulin resistance.

The lower IGFBP7 level in acromegaly patient cause diabetes mellitus. IGFBP7 is a promising new marker in acromegaly that should be investigated further.

## Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine/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 in University of Baghdad.

## Author's Contribution Statement

L.O.F. Conceptualization, analysis, visualization, curation of data, study, writing – original draft, B. A. A. Conceptualization, testing, project management, data analysis, writing-review &

editing, resources, visualization. K. G. J. and, I. N. S. Conceptualization, analysis, resources, visualization, writing the manuscript.

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## IGFBP7 علامة تشخيصية جديدة في امصال المرضى العراقيين المصابين بتضخم الأطراف

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### الخلاصة

كان الغرض من هذه الدراسة هو قياس مستويات مصلى البروتين المرتبط بعامل النمو الشبيه بالأنسولين (IGFBP7)، وعامل النمو الشبيه بالأنسولين 1 (IGF-1)، وهرمون النمو (GH)، والإنترلوكين 6 (IL-6) والأنسولين. في مرضى تضخم الأطراف والأصحاء. مجموعة المرضى تكونت من 60 مريضاً، بينما مجموعة الأصحاء تكونت من 30 شخصاً سليماً. تم تحديد تركيز IGF-1 و IGF-1 و GH و IL-6 والأنسولين. تشير نتائج الدراسة الحالية إلى أن مستوى IGF-1 في مجموعة المرضى كان أقل بشكل ملحوظ (1.690.07 نانوغرام / مل) مقابل 2.740.12 نانوغرام / مل، على التوالي،  $(p = 0.001)$ . كانت تراكيز IGF-1 و GH و IL-6 والأنسولين أعلى بشكل ملحوظ في مرضى تضخم الأطراف، وكانت الدقة التشخيصية (2.194) ممتازة في فصل ضخامة النهايات عن مجموعة المرضى الأصحاء، والمنطقة الواقعة تحت المنحنى ( $AUC = 0.871$ ،  $p = 0.001$ ). من نتائج الدراسة الحالية يمكننا أن نستنتج أن مصلى IGF-1 يمكن استخدامه لتمييز بين مرضى تضخم الأطراف عن الأفراد الأصحاء.

**الكلمات المفتاحية:** تضخم الأطراف، هرمون النمو، أنسولين، عامل النمو الشبيه بالأنسولين 1، البروتين المرتبط بعامل النمو الشبيه بالأنسولين.