Challenges Confounding Biochemical Diagnosis of Acromegaly

Mohd Zakwan Md. Muslim¹, Tuan Salwani Tuan Ismail¹, Siti Nadirah Ab Rahim²*, Rahidatul Fairuz Ibrahim³

¹Department of Chemical Pathology, Hospital Universiti Sains Malaysia, 16150 Kota Bharu, Malaysia
²Pathology Unit, Faculty of Medicine and Defence Health, National Defence University of Malaysia, Kem Perdana Sungai Besi 57000 Kuala Lumpur, Malaysia
³Hospital Pakar Sultanah Fatimah, Ministry of Health, Jln Salleh 84000 Muar, Johor

Corresponding author: nadirah@upnm.edu.my

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Abstract

Acromegaly is a disorder caused by growth hormone (GH) hypersecretion resulting in excessive release of insulin-like growth factor-1 (IGF-1). Both GH and IGF-1 measurements are crucial in its accurate diagnosis and in differentiating it from pseudoacromegaly. Early diagnosis is frequently restricted by its subtle, unnoticeable pathological changes; hence, clinical presentation is often late. Therefore, biochemical diagnosis is critical. However, the straightforward interpretation of these markers is hindered by factors that contribute to GH–IGF-1 discordance observed both in static and in dynamic function tests. These factors include both markers biological variation and their analytical assay limitations, which lead to false positive and negative results. This review is describing the pitfalls and challenges confounding the biochemical diagnosis of acromegaly. Despite these interpretive challenges, we strongly believe that interpretation of these results could be facilitated by effective clinician-laboratory professional communications which is the highlight of this review.

Keywords:
Acromegaly, Growth Hormone (GH), Insulin-Like Growth Factor-1 (IGF-1), IGF-Binding Proteins (IGFBP)

Introduction

Acromegaly is a slow and progressive disorder caused by persistent hypersecretion of GH which leads to excessive hepatic production of IGF-1[1]. Diagnosis of acromegaly is based upon combination of clinical examination as well as biochemical investigations of autonomous GH hypersecretion and elevated IGF-1 level. The global prevalence is 5.9 per 100,000 people while the incidence rate is 0.38 cases per 100,000
people \[2, 3\]. Due to its insidious onset, confirmation of diagnosis is usually made only after 4 to 7 years following GH hypersecretion \[3\]. Clinical manifestations are often the result of excessive IGF-1 production causing uncontrolled anabolic processes and tissue growth \[4\]. Typical presentations include facial changes, acral enlargement, and concurrent comorbidities like diabetes mellitus, hypertension, heart disease, and obstructive sleep apnoea (OSA) \[5\]. Compressive symptoms such as headache and bilateral hemianopia give clues to GH-secreting pituitary adenoma \[6\]. Meanwhile, some patients do not present with apparent acromegalic features, thus the resulting diagnostic delay increases risk of morbidity and mortality \[5\].

Contrarily, the presence of acromegaloid features without GH/IGF-1 axis abnormality is known as pseudoacromegaly \[6\]. This could be attributed to several endocrine causes (insulin resistance and hypothyroidism), genetic disorders (pachydermoperiostosis and multiple neuroma syndrome) or drug-induced reasons such as in Minoxidil and Phenytoin used \[7\]. It is postulated that acromegalic presentation in insulin resistance triggers a supraphysiologic insulin mitogenic signalling pathway that stimulates cellular growth \[8\]. In hypothyroidism, acromegalic features are the result of excessive mucopolysaccharides deposition \[9\]. Meanwhile, mutations in the gene responsible for 15-hydroxyprostaglandin dehydrogenase that causes an elevation in PGE-2 lead to tissue remodelling and vascular growth in pachydermoperiostosis \[9\].

Measurement of IGF-1 level is the key factor in the diagnosis and monitoring of acromegaly, but basal and nadir GH following glucose suppression test (GST) are also prudent \[5\]. However, there are limitations in the measurement of both markers that hinder a straightforward diagnosis.

**Biochemical markers in the diagnosis of acromegaly**

Guidelines recommend elevated IGF-1 as a screening test for suspected individuals. When IGF-1 is elevated, GH level reaching more than 1 mcg/L following an oral glucose tolerance test (OGTT) is diagnostic \[3, 10\]. With advances of highly sensitive assays that allow the detection of GH nadir as low as <0.3 mcg/L, GH nadir of above 0.4 mcg/L in OGTT is recommended in the diagnosis of acromegaly, especially in the context of raised IGF-1 but low GH level (<1 mcg/L) \[9, 10\]. In normal individuals, the GH nadir value during an OGTT is undetectable as secretion is suppressed. However, a high GH value owing to the lack of suppression is suggestive of acromegaly \[3\]. Nonetheless, considering factors like biological variability, the response of these analytes to dynamic testing, and their analytical assay limitations, such expected biochemical result might not be observed.

**IGF-1 measurement**

A raised IGF-1 level is proven to correlate well with excessive production of GH \[11\]. However, unlike GH, IGF-1 is secreted constantly, thus it is not affected by pulsatility \[3\]. It stays longer in circulation with a 22-24 hours half-life compared to only 10-14 minutes for GH \[12, 13\]. Therefore, IGF-1 is measurable at any time throughout the day. Besides, fasting blood sampling is not required hence convenient for both patients and clinicians \[6, 12\].

Nevertheless, IGF-1 measurement is influenced by many factors. First, it should be interpreted using age and gender-specific reference ranges \[6, 14\]. This is because circulating IGF-1 is maximal during peripubertal and declines with age but varies between genders \[14\]. Before the age of 45 years, serum IGF-1 level in men is lower than that of women, and at 45-49 years it reaches similar levels in both genders. Men’s IGF-1 level then peaks above that of women’s value after the age of 50 years and remains higher thereafter \[15\]. Physiological elevations are also observed in pregnancy and during puberty thus, any suspicion of acromegaly should be reconfirmed with subsequent measurements \[11\]. In pregnancy, a false-positive IGF-1 result is due to a significant increase in placental GH secretion. It induces hepatic IGF-1 secretion causing...
an increment of IGF-1 up to 2-3 folds in the second half of pregnancy, which then peaks at 37 weeks [11]. A linear relationship between IGF-1 and GH exists up to GH value of 20 to 60 mcg/L but subsequently it plateaus, postulated to be due to hepatic GH receptor saturation [9]. Consequently, IGF-1 beyond that level should be interpreted with caution. False negative results could also be found in malnutrition, liver failure, renal failure, and oral oestrogen use (table 1) [16].

Analytically, heterophilic antibody interference could result in a false negative IGF-1 result [17]. Available IGF-1 assays include radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and immunochemiluminescence assay (ECLIA) [17, 18]. Assay development for the measurement of IGF-1 originally focused on factors such as availability of antisera with high affinity and high specificity, and availability of pure peptide standard [18]. The conventional competitive assays are the radioimmunoassays (RIAs), which are prone to binding protein interference. This has been largely resolved by current assays which uses extraction method such as acidification to remove any interfering IGF-binding proteins (IGFBP) [19]. Dilution and the use of antibodies with high affinity and high specificity toward IGF-1 further lower the risk of IGFBP and IGF-II interference [20].

Table 1: False positivity and false negativity of IGF-1 result.

<table>
<thead>
<tr>
<th>False positive</th>
<th>False negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>Malnutrition</td>
</tr>
<tr>
<td>Puberty</td>
<td>Liver failure</td>
</tr>
<tr>
<td>Micromegaly</td>
<td>Renal failure</td>
</tr>
<tr>
<td></td>
<td>Oral oestrogen</td>
</tr>
<tr>
<td></td>
<td>Critical illness</td>
</tr>
</tbody>
</table>

Note: False positive and negative IGF-1 will interfere with suppression of GH during GST.

Growth hormone (GH) measurement

Single GH measurement

Confirmatory GH measurement should follow an elevated or equivocal IGF-1 level [21, 22]. However, a single basal GH value measured after a period of fasting will usually be elevated. Furthermore, GH pulsatility and marked variability further limit its single measurement [9]. Its nadir value can reach as low as below the detectable limit and is prone to biological response to stress, physical exertion, fasting state, and several pathological conditions such as poorly controlled diabetes mellitus, renal failure, and malnutrition [6]. Additionally, GH value could be seen normal in mild or early acromegalic stages. These patients usually have a low but sustained GH secretion causing a high IGF-1 value [9]. Meanwhile, random GH levels are also overlapping between healthy subjects and acromegalic patients [13].

Mean 24-hour GH measurement

Since the basal or random GH level is inconclusive for diagnosis, mean GH measurement is an alternative. It could be obtained from a frequent sampling of GH over 24-hour or a shorter 8-9-hour-duration [13]. However, studies demonstrated that, the mean 24-hour GH value in acromegaly patients overlaps with that of healthy subjects, hence the difficulty in ultimately distinguishing between the two [13, 21].

Dynamic function tests

The commonly used dynamic function test in the assessment of acromegaly is the OGTT while the thyrotropin-releasing hormone (TRH) stimulation test is the alternative [23]. In OGTT, glucose introduction stimulates hypothalamic neuropeptides (like somatostatin) that suppress GH secretion [24]. After at least 8-hour of fasting, blood sampling is performed at baseline and every 30 minutes within 120 minutes.
following a 75g glucose consumption. The lack of GH suppression is diagnostic [25, 26]. The shortcoming to the interpretation of this test is a paradoxical response seen following an oral glucose load in the presence of insulin resistance [27]. Some patients may exhibit elevated IGF-1 but show normal GH levels (relatively suppressed GH post-OGTT) [28, 29]. Contrarily, for patients with low IGF-1 but high GH level, the possibility of a false-negative IGF-1 must be investigated. An example includes insulin resistance that leads to the downregulation of IGF-1 production by the liver and other tissues [30]. Another example includes hypothyroidism which causes an elevation in Insulin-like growth factor binding proteins (IGFBPs) interfering with IGF-1 measurement (table 2) [31]. It is said that most IGFs (~75%) circulate as a 150kDa ternary complex containing high-affinity IGF that is bound to IGFBP3, and a glycoprotein called acid labile subunit (ALS). These IGF-IGFBP complexes interfere with IGF-1 analytical measurements leading to a falsely low level [32, 33]. Meanwhile, in liver disease, low IGF-1 is postulated to be due to a decrease in GH receptors on the liver and a progressive reduction of liver synthesis capability [34]. Increased urinary losses of serum IGF-IGFBP complexes in kidney failures appear to result in low levels of IGFBPs and IGF-1 [35]. For drugs like oral oestrogen, it inhibits the metabolic action of GH in the liver, causing a fall in IGF-1 production [36].

A recent study demonstrated that nadir GH following OGTT differs in stratified groups [37]. For example, normal body mass index (BMI) individuals have significantly higher mean GH than overweight and obese individuals (0.124 and 0.065 mcg/L respectively, \( P = 0.0317 \)) [37]. Therefore, using the general cut-off might be misleading, especially in the latter group. It has been proposed that adjustment of the GH cut-offs for OGTT based on sex and BMI increases its sensitivity in the detection of acromegaly [3, 37]. In addition, a major analytical factor includes the lack of comparability among various commercially available GH measurement assays. This leads to the variation in GH cut-offs. GH assay is principally a non-competitive immunometric assay with a sensitivity of 0.2 mcg/L to as low as 0.002 mcg/L in the newer high-sensitivity assay [37, 38]. Circulating GH in the human body is a mixture of several molecular isoforms. The most abundant isoform is a 22kD GH molecule, followed by a smaller 20kD molecule [39]. Because of its molecular heterogeneity, assay standardization is hard to achieve. Polyclonal antisera are not specific. Cross-reactivity between placenta hGH leads to an increase in GH levels during pregnancy [40]. Another analytical challenge in measuring GH is the impact of Growth hormone-binding protein (GHBP). GHBP can cause steric hindrance in GH assays; therefore, when present in excess leads to an underestimation of GH concentration [41].

Endocrine Society Clinical Practice Guideline proposes the lack of suppression of GH below 1 mcg/L (and elevated IGF-I for age and sex) for diagnosing acromegaly [3]. The prevalence of acromegaly and the percentage of remission largely depend on the cut-off values used. Specific GH assays calibrated against the latest recombinant standard indicate that the cut-off of 1 mcg/L is inappropriately high, suggesting that assay standardization or at least commutability is paramount [39]. Hence, several studies have now recommended a nadir GH above 0.4 mcg/L using ultrasensitive assays as the diagnostic cut-off [4, 38]. Nevertheless, despite the assay-related differences in the published cut-offs, most guidelines do not specify the assay used to generate the stated GH cut-off levels [39]. Therefore, consultation with laboratory professionals is vital to ensure the diagnostic GH cut-off used is in conformity with the analytical measurement assay [4].

In the TRH stimulation test, 200 ug of intravenous TRH is administered to patients with blood sampling performed at baseline, followed by sampling every 30 minutes until 120 minutes. In GH-producing pituitary adenomas, GH responsiveness to TRH is observed. This happens because of an abnormality in the sensitization of TRH receptors towards pituitary somatotroph adenoma cells [42]. By calculating the TRH ratio (the peak to basal GH ratio), those with a ratio higher than 2 are regarded as TRH responders.
Nevertheless, this response is again, not unique to acromegaly because it has been observed in conditions like depression and liver cirrhosis \[43\].

Table 2: Causes of false positive and false negative GH levels following GST and the reasons.

<table>
<thead>
<tr>
<th>Causes of false positive result</th>
<th>Reasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistance</td>
<td>Reduce GH receptor expression on hepatocytes, limit IGF-1 production, thus reducing or loss of negative feedback inhibition of GH production.</td>
</tr>
<tr>
<td>Liver disease</td>
<td>Increase urinary losses of serum IGF-IGFBP complexes, thus reduce IGF-1 level, which subsequently reduces or negative feedback inhibition of GH production.</td>
</tr>
<tr>
<td>Renal failure</td>
<td>Interfere with the metabolic activity of GH in the liver, thus reducing IGF1 production, which subsequently reduces or negative feedback inhibition of GH production.</td>
</tr>
<tr>
<td>Oral oestrogen</td>
<td>Low IGF-1 in nutritional deficiency individuals secondary to the number of hepatic growth hormone receptors.</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>Reduced level of IGF-1 during critically ill episodes causes less negative feedback inhibition of GH production.</td>
</tr>
<tr>
<td>Critical illness</td>
<td>Increase placental GH secretion during pregnancy IGF-1 is maximal during puberty and declines with age.</td>
</tr>
<tr>
<td>Pregnancy, puberty</td>
<td>Elevated IGFBP in the blood interfere with IGF-1 analysis.</td>
</tr>
</tbody>
</table>

Differentiation of acromegaly from pseudoacromegaly is challenging with discordant findings obtained with the measurement of GH and IGF-1 levels. Examples include patients with elevated IGF-1 with normal GH levels in micromegaly and a normal/low IGF-1 level with raised GH levels in the case of acute critical illness, oral estrogen use, and uncontrolled diabetes \[6\]. The pathologic mechanism of the discordance between GH and IGF-1 in micromegaly is still unknown \[39\]. Conversely, during an acute critical illness, IGFBP-3 level increases, while IGFBP-1 level decreases along with protease activity. This fall is thought to be due to critical illness-associated protease activity, which leads to a raised in IGF-1 clearance rates. Another possible explanation for the reduction of circulating IGF-1 levels found during critical illness is that nutritional deficiency leads to alterations in cytokine and endotoxin activity, therefore, decreasing the number of hepatic GH receptors \[44\]. Due to these variabilities, biochemical changes should always be interpreted with reference to clinical presentation.

**Conclusion**

Measuring IGF-1 level as an initial test is a critical factor in the diagnosis of acromegaly. It is a measure of the biological activity of the disease and peripheral response to circulating GH. Diagnosis confirmation requires a raised IGF-1 coupled with GH suppression following OGTT. However, early diagnosis of acromegaly and distinguishing it from pseudoacromegaly is challenging due to various factors that could lead to discordant IGF-1-GH results; namely biological variations, response of markers towards dynamic function test and several analytical assay shortcomings. Therefore, to ensure accurate clinical
interpretation and its conformity with the analytical assay used, clinician communication with laboratory professionals is prudent.

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Conflict of Interest Disclosure
None to declare

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