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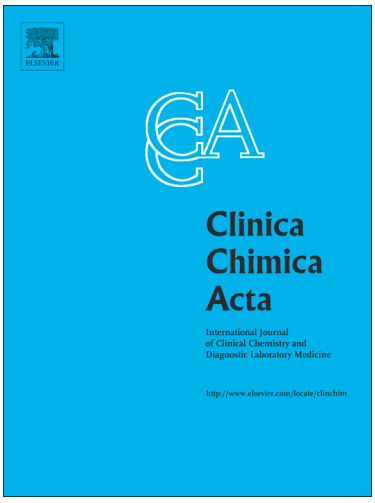

Case report

Macro-GH – a clinical entity causing a diagnostic challenge – a case report

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Macro-GH – a clinical entity causing a diagnostic challenge – a case report.

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Abstract

Aim

Presentation of a new case of a patient with macro-GH, that may interfere with different GH assays leading to false-positive results in serum samples.

Case presentation

A 61-year-old female was referred with a pituitary macroadenoma and elevated growth hormone levels The laboratory tests showed increased fasting GH level, measured by a sandwich chemiluminescence immunoassay (LIAISON® XL) without suppression on oral glucose tolerance test and normal IGF-1. The patient did not have the typical signs and symptoms of acromegaly. The patient underwent a transsphenoidal resection of a pituitary tumor, showing only α-subunit immunostaining. Postoperative GH levels remained elevated. An interference in the determination of GH level was suspected. GH was analyzed by three different immunoassays, UniCel DxI 600, Cobas e411 and hGH-IRMA. Heterophilic antibodies and rheumatoid factor were not detected in serum sample. GH recovery after precipitation with 25% polyethylene glycol (PEG) was 12%. Size-exclusion chromatography confirmed the presence of macro-GH in serum sample.

Conclusion

If results of laboratory tests are not consistent with the clinical findings, the presence of an interference within immunochemical assays could be suspected. To identify interference caused by the macro-GH, the PEG method and size-exclusion chromatography should be used.

Key words: Macro-GH, macrocomplex, interference, immunoassay, acromegaly, macroadenoma.

Abbreviations: GH, growth hormone; IGF-1, insulin-like growth factor 1; CLIA, chemiluminescent immunoassay; ECLIA, electrochemiluminescent immunoassay; IRMA, immunoradiometric assay; PEG, polyethylene glycol; SEC, size-exclusion chromatography.

1. Introduction

Determination of the concentration of growth hormone (GH) in blood is necessary in the diagnosis and monitoring of treatment in patients with acromegaly or with GH deficiency. In acromegaly, GH secretion is increased resulting in higher baseline GH and insulin-like growth factor 1 (IGF-1) levels [1]. Chemiluminescence immunoassay (CLIA) and electrochemiluminescence immunoassay (ECLIA) are the most popular methods used for diagnostics in clinical laboratories.

We present here a patient with pituitary macroadenoma, without clinical features of acromegaly, in whom high GH level was found due to the occurrence of a rare type of assay interference, the presence of GH macromolecules (macro-GH). We suggest that the various routinely available laboratory methods can be used to identify potential causes of interferences. In this case, we performed polyethylene glycol (PEG) precipitation as a screening method to

confirm the presence of macro-GH as an interfering factor, and this was confirmed using the reference method, size-exclusion chromatography (SEC).

1. Case presentation

A 61-year-old female was referred to our department with suspected acromegaly. She had increased random GH levels of 8.2-9.0 µg/L [reference range 0,1- 6,88 µg/L], normal IGF-1 of 144 µg/L [reference range for her age 94-229 µg/L], secondary adrenal insufficiency (8:00 am cortisol 143.5 nmol/L), hyperprolactinemia (34.8 µg/L), and bitemporal visual field defect. A pituitary MRI showed a 13x12x15 mm pituitary macroadenoma compressing the optic chiasm. Her medical history included epilepsy treated with anti-epileptic drugs for many years. The patient did not have the typical signs and symptoms of acromegaly and her BMI was 27 kg/m^2. The laboratory test, using the LIAISON® XL analyzer (DiaSorin, Italy), showed increased fasting GH level without suppression on oral glucose tolerance test (GH nadir 6.6 µg/L) and normal IGF-1 of 144 µg/L [reference range for her age: 94-229 µg/L]. As active acromegaly with normal circulating IGF-1 has been described in the literature [2], a trial 3-month treatment with a long-acting somatostatin receptor ligand (Somatuline Autogel 120 mg/monthly) was initiated. Despite somatostatin receptor ligand treatment, elevated GH (10.4 µg/L) with normal IGF-1 (142 µg/L) levels persisted. No visible decrease in pituitary adenoma size on MRI was detected. The patient underwent a transsphenoidal resection of a pituitary tumor. The immunostaining was positive for α-subunit and negative for GH, PRL, ACTH, TSH, FSH and LH with a Ki67 index <1%, suggesting a silent gonadotroph adenoma. Eight months and 20 months after the surgery, laboratory tests remained unchanged with elevated GH level 25.1 and 16.1 µg/L and normal IGF-1 178 µg/L [43-241 µg/L]. Based on the clinical presentation, laboratory tests and immunostaining of the pituitary adenoma an interference in the determination of GH level was suspected.

A random postoperative serum sample was tested for GH concentration on the original LIAISON® XL (CLIA) platform using monoclonal antibodies, as well as three other platforms: UniCel DxI 600 (Beckman Coulter, UK; CLIA, polyclonal antibody), Cobas e411 (Roche Diagnostics, UK; ECLIA, monoclonal antibody) and hGH-IRMA (immunoradiometric assay, DiaSource, Belgium, monoclonal antibody). The Beckman Coulter, Roche and DiaSource assays showed GH concentration within the reference range for the given method, while the LIAISON method showed the previously observed high level of GH (Table 1).

The linearity in serial dilutions was then tested. The serum sample collected from the patient was diluted in 1/2, 1/4 and 1/8 ratios. GH recovery in diluted samples showed an increase in GH concentration with increasing dilution factor (non-linearity). The recovery was 114% (neat 16.1 µg/L; 8x dilution 18.4 µg/L) for the LIAISON® XL.

In the next step, the test sample (16.1 µg/L, LIAISON® XL) was analyzed with heterophile-antibody blocking tubes (Scantibodies, USA). The post-incubation GH recovery was 95%, indicating no interference from heterophilic antibodies. The absence of rheumatoid factor in serum was also demonstrated.

In order to check whether an excess of anti-GH antibodies was present in the sample, that could sequester GH of another patient who indeed has acromegaly, the patient's serum (16.1 µg/L, LIAISON® XL) was incubated for 4 hours with serum from another patient serum with confirmed acromegaly and high GH level (11.5 µg/L) in the ratio of 1:1. The post-incubation recovery of GH was 98% (expected GH value13.8 µg/L; measured value 13.5 µg/L (LIAISON® XL). The decreased GH recovery did not clearly indicate an excessive binding of exogenous GH by autoantibodies.

Next, the patient serum was precipitated with 25% PEG (PEG 6000, Sigma Aldrich). As a result of this analysis, the GH recovery was 12%, which indicated that 88% of GH was in form of macrocomplex (Table 1).

Finally, in order to confirm the diagnosis of macro-GH as an interfering factor, patient sample was analyzed using the size-exclusion chromatography reference method. Ultimate 3000 HPLC system (Dionex, USA) equipped with an autosampler with fraction collector and DAD detector connected to a Superdex® 200, 10 × 300 mm column (GE Healthcare Europe GmbH, Freiburg, Germany) was used. The GH peak was found at the front of the elution in the high molecular weight fraction in the range of 670-158 kDa, confirming the presence of macro-GH (Fig 1C). Sera from patients with high GH concentration (Fig 1A) and GH concentration in the reference range (Fig 1B) were used as controls. The GH-derived peak was in the range of 44-17 kDa, which corresponds to a GH molecule with a molecular weight of 22 kDa.

2. Discussion

Interferences in immunoassays are rare (0.4-4.0%) but should be taken into account as they can cause misdiagnosis and lead to inappropriate treatment [3].

Immunoassays (CLIA, ECLIA) differ from each other in terms of format (competitive and non-competitive), label detection system and antibody type (monoclonal and polyclonal) used in the test [4]. Although immunoassays are quite robust, they are still susceptible to various types of interferences [5].

Interferences in immunoassays caused by hormone macrocomplexes are most often described in relation to macroprolactin [6], and much less frequently to macro-TSH [7-14], macro-FSH [15], macro-LH [16] or macro-hCG [17]. Just like PRL and other proteins, GH undergoes post-translational modifications, resulting in various forms of this hormone. There are three major forms of this hormone: little GH with a mass of 20-22 kDa (which is dominant in the circulation), big GH >45 kDa and big-big GH >60 kDa. Big-big GH, similarly to big-big PRL, is a macrocomplex that was created as a result of the aggregation of monomeric forms or the binding of a free hormone molecule with antibodies or plasma proteins. In healthy people, big GH and big-big-GH make up to 30% of total GH [18,19]. To date, few cases of GH macromolecule interfering with GH determinations in some patients have been reported [20-22]. We present the new case of a patient with GH macrocomplex that resulted in an interference in immunoassay.

In our case, when looking for interference, we decided to rely on the combination of detection algorithms proposed by Favresse [23] and Loh [7]. We have performed several previously described tests, the key among these are PEG precipitation and size-exclusion chromatography.

PEG precipitation is a recognized screening method for detection of hormone macromolecules, especially macroprolactin. In our patient, the GH recovery after PEG precipitation was only 12%, which is a similar result obtained with other macromolecules described by various authors. For TSH, recovery varied from 3.2% to 24% [7,8,11,13]. For FSH, LH and hCG recovery of the free hormone was 24%, 3% and <5%, respectively [15,16,17]. Although this method has numerous advantages, such as speed of execution, simplicity, low cost, and high availability, it should be remembered that it also has some limitations. Firstly, PEG may precipitate some smaller forms of hormones (e.g., free, or monomeric form of the tested hormone). The higher the molecular weight of a protein, the more susceptible it is to PEG precipitation. Clearly, the dominant immunoglobulin in human serum is IgG. In the literature,

macrohormone compounds are usually described as a complex of a free hormone and an IgG immunoglobulin (less frequently IgM and IgA). This leads to another limitation of this method – the fact that PEG precipitation does not remove the entire hormone-IgG complex or the IgG itself. Therefore, different cut-off points are used for interpretation of the test results for different hormones that can form macromolecules e.g., macro-PRL recovery % is <50, while for macro-TSH it is <10%-20% [8,18,24]. PEG precipitation may also cause false positive results in patients with increased levels of gamma globulins or with HIV [25]. In addition, this technique is not specific for IgA and results in only partial precipitation of this immunoglobulin, which may lead to the overlooking of macrocomplexes that are associated with IgA [14].

Size-exclusion chromatography or gel filtration chromatography is a reference method for detecting macromolecules. This technique provides a well-defined separation of analytes and a short analysis time. Unfortunately, it is also expensive, time-consuming and requires highly qualified professionals. In our case the macro-GH peak was found in the high molecular weight fraction in the range of 670-158 kDa, confirming the presence of a macrocomplex. This "gold standard" method was also used to identify macro-TSH. Various authors demonstrated that the peak from TSH and IgG complex was at the region corresponding to the molecular size of IgG (>150 kDa) [7,8,10,12,13]. Moreover, size-exclusion chromatography is decisive when PEG precipitation gives false negative [14] or positive result [11]. There are also reports of other hormones such as FSH, LH and hCG that were found in the macro form when size-exclusion chromatography was performed [15,16,17].

3. Conclusions

If elevated GH is not consistent with the patient's clinical presentation and other hormone results, interference in immunoassay should be suspected. In this case, a number of tests should be performed that would allow the identification of the interfering factor. We propose to carry

out hormone assays using two or three different immunoassays, serial dilution test, incubation of the test sample with inactivating heterophile antibodies (e.g., heterophile-antibody blocking tubes), precipitation of the sample with 25% PEG and, finally, identification of the interfering factor by the reference method, size-exclusion chromatography (SEC). These tests will be particularly helpful in case of a macromolecule (macrocomplex) behaving as an interfering factor, in our case macro-GH.

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Declarations of Interest

none.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Authors' Contributions

SBM: Conceptualization, Writing - Original draft. MO: Conceptualization, Investigation. Writing - Original draft, Supervision, Writing - Review&Editing. GT: Formal analysis, Resources. KK: Investigation, Resources. KM: Writing - Review&Editing. KR: Investigation, Resources. ZW: Writing - Review&Editing. GP: Supervision, Investigation, Writing - Review&Editing. All authors approved the final version of manuscript.

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Table 1. Serum GH results determined with different immunoassays and using the 25% PEG method.

GH levels with different immunoassays				
Assay	GH (reference range)	Method	Assay Antibodies	Intra-assay variability
LIAISON® XL (DiaSorin)	16.1 (0.1-6.88)	CLIA	monoclonal antibodies (mouse)	4.4%
UniCel DxI 600 (Beckman Coulter)	3.7 (0.010-3.807)	CLIA	Polyclonal antibodies (goat)/ monoclonal antibodies (mouse)	2.1%
Cobas e411 (Roche)	1.9 (0.126-9.88)	ECLIA	monoclonal antibodies (mouse)/polyclonal antibodies (sheep)	2.3%
hGH-IRMA (DiaSource)	1.5 µIU/mL (<0.2-10)	IRMA	monoclonal antibodies	4.4%

GH levels before and after precipitation with PEG (LIAISON® XL)			
	GH before PEG (µg/L)	GH after PEG (µg/L)	GH recovery (%)
Control sample 1	12.5	10.0	80
Control sample 2	26.3	21.2	81
Control sample 3	3.6	3.1	86
Control sample 4	6.8	6.2	92
Test sample	16.1	2.0	12

Abbreviations: CLIA, chemiluminescent immunoassay; Control sample 1-4, samples from patients diagnosed with acromegaly were used as controls for PEG precipitation; ECLIA, electrochemiluminescent immunoassay; GH, growth hormone; IRMA, immunoradiometric assay; PEG, polyethylene glycol; Test sample, patient's sample under the investigation.

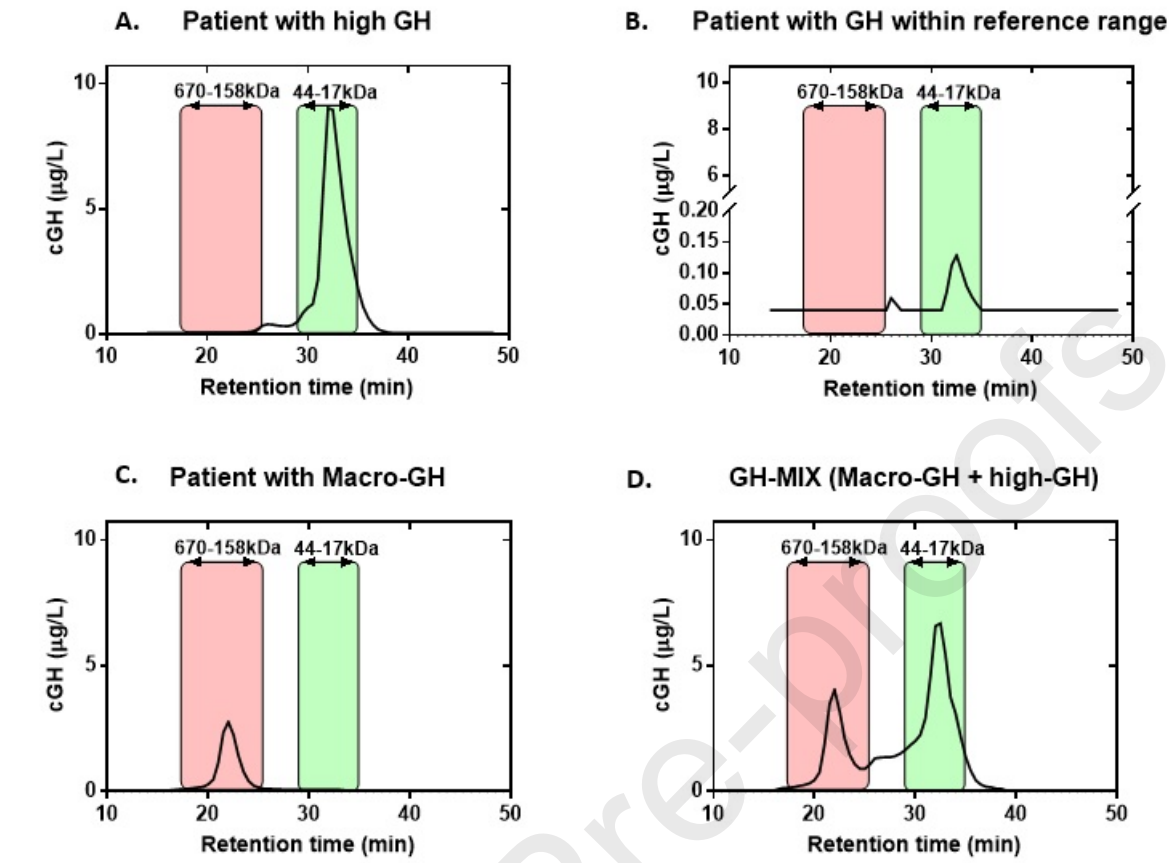

Fig. 1. Size-exclusion chromatogram of sera from control patients with (A) high GH concentration (115 µg/L) and (B) with GH concentration within reference range (2.46 µg/L) (C) the patient sample showing presence of macro-GH and, (D) mixed sera from the patient with macro-GH and high GH concentration