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Olgu Sunumu / *Case Report*

Prolactin Elevation Due to Heterophile Antibody Interference in Pituitary Deficiency

Hipofiz Yetmezliğinde Heterofil Antikor İnterferansı Nedeniyle Prolaktin Yüksekliği

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ABSTRACT

This case highlights the effect of heterophilic antibody interference in two siblings with hypopituitarism. Prolactin measurements performed on the UniCel DxI 800 in patients followed up with a diagnosis of hypopituitarism were found to be falsely elevated. For further investigation, polyethylene glycol precipitation (PEG), heterophilic antibody blocking tubes (HBT), serial dilution, and three different blocking antibodies were used. The PEG test showed a reduction of less than 40%, and macroprolactinemia was excluded. In the serial dilution test, the results were not linear, raising suspicion of interference. Lower prolactin levels were reported in three different systems. After HBT application, prolactin levels unexpectedly increased in both siblings. The increase in prolactin levels observed after HBT and the addition of different blocking antibodies could be due to an unexpected interaction between the blocking antibodies and the analysis reagents. The investigations using blocking antibodies were unable to determine the exact nature of the interfering agent.

Key Words: Prolactin, Hyperprolactinemia, Heterophil Antibodies, Interference, Hypopituitarism

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ÖZET

Bu vaka, hipopituiterizmli iki kardeşte heterofilik antikor interferansının etkisini vurgulamaktadır. Hipopituiterizm tanısı ile takip edilen hastalarda UniCel DxI 800 üzerinde yapılan prolaktin ölçümleri yanlış şekilde yüksek bulunmuştur. İleri inceleme için polietilen glikol çökelmesi (PEG), heterofilik antikor engelleyici tüpler (HBT), seri dilüsyon ve üç farklı blokan antikor kullanılmıştır. PEG testi, % 40'ın altında bir azalma göstermiş ve makroprolaktinemi dışlanmıştır. Seri dilüsyon testinde, sonuçların doğrusal olmaması nedeniyle interferans şüphesi ortaya çıkmıştır. Üç farklı sistemde ise daha düşük prolaktin seviyeleri raporlanmıştır. HBT uygulaması sonrası prolaktin seviyeleri her iki kardeşte de beklenmedik şekilde artmıştır. Farklı bloke edici antikorların eklenmesinden sonra da prolaktin sonuçlarında görülen artış, bloke edici antikorlar ile analiz reaktifleri arasındaki beklenmedik etkileşimden kaynaklanabilir. Blokan antikorlarla yapılan incelemeler interferans ajanının tam doğasını belirleyememiştir.

Anahtar Sözcükler: Prolaktin, Hiperprolaktinemi, Heterofil Antikorlar, İnterferans, Hipopitüitarizm

INTRODUCTION

Hormone immunoassays can be significantly influenced by the presence of heterophile antibodies, human anti-animal antibodies, autoantibodies, and rheumatoid factors, which can lead to inaccurate hormone measurements. These interferences compromise assay precision by interacting with antibodies or detection labels, such as streptavidin or alkaline phosphatase (ALP), used in the reagents. In enzyme-based immunoassays, the presence of enzyme inhibitors or activators can also alter the signal, resulting in altered test results (1-4). Hypopituitarism is a rare condition characterized by a deficiency in one or more hormones produced by the pituitary gland. The most common cause of hypopituitarism is pituitary tumors, which account for 61% of cases. These tumors may lead to the overproduction of a specific hormone while simultaneously causing deficiencies in others. Hypopituitarism is associated with an increased risk of mortality, particularly due to higher rates of cardiovascular and respiratory diseases, underscoring the importance of early diagnosis and intervention. Accurate hormone measurements are especially critical in managing conditions like hypopituitarism, where precise hormone monitoring is essential (5-7). Here, we present the case of two siblings undergoing hormone assessments for hypopituitarism, where interference in the assays resulted in inconsistent prolactin levels across different immunoassay platforms.

CASE PRESENTATION

The two siblings, aged 18 (patient 1) and 11 (patient 2), have been receiving growth hormone (GH) replacement therapy for the past three years due to hypopituitarism. During this period, their prolactin levels were measured seven and nine times, respectively, using the Infinity c 8000 analyzer (Roche Diagnostics, Penzberg, Germany), consistently showing low results (<0.094 ng/mL for both). Additionally, both siblings exhibited low GH responses following the Clonidine GH Stimulation Test. GH levels, measured on the same Infinity c 8000 analyzer, did not exceed 7 ng/mL, confirming GH deficiency (8). However, when testing was switched to the UniCel DxI 800 (Beckman Coulter, Brea, CA, USA), significantly elevated prolactin levels were observed in both siblings: 41.22 ng/mL for patient 1 and 27.72 ng/mL for patient 2. These high prolactin values were inconsistent with both the previous results and the clinical diagnosis, prompting further investigation. Informed consent was obtained to participate in the study and a new sample was drawn for re-evaluation

Suspecting the presence of heterophile antibodies, further studies were conducted. The patients were called and invited to the

laboratory. Prolactin levels were re-measured on the UniCel DxI 800 analyzer. The detection limit of prolactin assay was 0.25 ng/mL, and the total coefficient of variation (CV) was 6.92%, 3.32%, and 4.23% for low, medium, and high prolactin concentrations, respectively. (catalog number: 472010). To remove potential interfering antibodies, the patients' sera underwent precipitation with polyethylene glycol (PEG) 6000 (product number: 29577, Merck Ltd.). Simultaneous analyses of both the control sample and the patients' sera were performed, and the percentage decrease in prolactin levels after PEG precipitation was calculated. The percentage decreases were below 40% for both and macroprolactinemia was excluded (Table 1). For further investigation of heterophile antibodies, heterophilic blocking tubes (HBT) (Scantibodies Laboratory, Inc., USA, catalog number:0257C) were utilized, comparing results with the original measurements. The HBT contains specific binders that deactivate heterophile antibodies in 500 µL of sample. Analyses were carried out simultaneously on a control sample and the patients' sera. A percentage change of 9.67 % was found for the control, which was acceptable, whereas prolactin levels were increased by 320.3% and 94.10% in patient 1 and patient 2 respectively. (Table 1) Dilution testing was conducted through serial dilutions (1/2, 1/4, 1/8, 1/16, and 1/32) using the manufacturer's zero calibrator and results showed the presence of interferences since the test was not linear (Table 2). Subsequently, samples were sent to other laboratories using the Advia Centaur XP (Siemens Healthcare Diagnostics, Tarrytown, USA), Alinity i 1000 (Abbott Laboratories, Diagnostic Division, Abbott Park, IL, USA), and Roche systems. All platforms returned low prolactin results consistent with the initial measurements from the Roche Infinity c 8000 analyzer. Additional tests, including ALP, rheumatoid factor (RF) and immunofixation analyses, showed that ALP and RF levels remained within the reference range, and no monoclonal peaks were found on serum immunofixation analysis. Further investigation was performed at the Beckman Coulter complaint handling unit laboratory, where three different blocking reagents were applied to the patients' sera. Pools 1 and 2 contained different blockers: PolyMak 33 and HBR-1 (Pool 1), and Goat, Mouse, Rabbit, Sheep, and Bovine IgGs (Pool 2), all animal-derived antibodies. Pool 3 (AP Mutein, Scavenger ALP), a pool of blocker related to ALP, was also tested. The percent changes in prolactin levels for patient samples ranged between -10.00% and +37.99% after the addition of the pools 1/2/3. A percent change between -25% and +25% is expected if no interference is present. For patient 2, percent changes above +25% were observed with Pools 1 and 3, suggesting that the blockers were ineffective in decreasing the prolactin results (Table 3). Since the changes were positive, these results could not be interpreted. The interference testing did not allow for the identification of the nature of the interference, as none of the blockers used decreased the signal, and other potential interfering substances may exist.

Table 1. PEG Precipitation and HBT Analysis of Patients and Control
Tablo 1. Hastaların ve Kontrollerin PEG Presipitasyonu ve HBT Analizi

	Neat Results (ng/mL)	PEG Results (ng/mL)	Percentage Change (%)	HBT Tube (ng/mL)	Percentage Change (%)
Patient 1	66.32	50.50	-23.85	128.73	+94.10
Patient 2	34.88	33.98	-2.58	146.61	+320.3
Control	6.23	4.93	-20.86	10.34	+9.67

Table 2. Serial Dilution Results of The Patients
Tablo 2. Hastaların Seri Seyreltme Sonuçları

	Dilution	Prolactin Results (ng/mL)	Percent Change (%)
Patient 1	Neat	66.32	-
	1:2 Dilution	62.45	-5.83
	1:4 Dilution	52.85	-20.28
	1:8 Dilution	40.98	-38.20
	1:16 Dilution	33.67	-49.23
	1:32 Dilution	30.83	-53.51
Patient 2	Neat	34.88	-
	1:2 Dilution	32.48	-6.88
	1:4 Dilution	28.12	-19.38
	1:8 Dilution	20.33	-41.71
	1:16 Dilution	18.02	-48.33
	1:32 Dilution	17.71	-49.22

Table 3. Observed Changes Using Pool 1, Pool 2, and Pool 3
Tablo 3. Havuz 1, Havuz 2 ve Havuz 3 Kullanılarak Gözlemlenen Değişiklikler

	Prolactin Results (ng/mL)	Percent Change (%)
Patient 1 (Neat)	56.76	-
Patient 1 + Pool 1	52.46	-7.57
Patient 1 + Pool 2	51.36	-9.51
Patient 1 + Pool 3	63.08	+11.13
Patient 2 (Neat)	30.78	-
Patient 2 + Pool 1	42.48	+37.99
Patient 2 + Pool 2	27.70	-10.00
Patient 2 + Pool 3	39.44	+28.12

Pool 1: PolyMak 33 and HBR-1,
Pool 2: Goat, Mouse, Rabbit, Sheep and Bovine IgGs,
Pool 3: AP Mutein, Scavenger ALP

DISCUSSION

This case underscores the significant impact of heterophile antibody interference on hormone immunoassays. In the presented cases of the two siblings, the presence of heterophile antibodies resulted in falsely elevated prolactin levels when tested on the UniCel DxI 800 analyzer while other platforms showed low levels. A comprehensive investigation, including PEG precipitation, serial dilution, and HBT tests were performed to confirm the presence of heterophile antibodies. However, analyses using different blocking antibodies could not allow for the identification of the nature of the interference.

Over the years, various interferences in immunoassays have been identified. While some of these interferences are now rarely encountered in routine practice, issues such as cross-reactions, heterophile antibodies, biotin, and anti-analyte antibodies continue to pose challenges. Additionally, as new therapies are developed, new types of interference are emerging adding complexity to immunoassay evaluations. The interference may have been caused by an exogenous substance, such as a drug or compound absorbed by the patients, or an endogenous factor, such as heterophile or anti-animal antibodies produced by the patients (9). Both siblings, undergoing growth hormone replacement therapy with

daily doses of recombinant human growth hormone (rhGH, Omnitrope, Genotropin) between 0.7 and 1.5 mg. According to the Beckman Coulter. Access Prolactin Reagent Kit Insert. it is stated: "No significant cross-reactivity was observed when recombinant human growth hormone (rhGH) was added to the Access Prolactin Calibrator S1 (2 ng/mL) at 10.82 IU/L." Given that the administered rhGH doses were lower than this concentration, interference related to rhGH treatment was considered unlikely.. Despite advances in our knowledge and understanding of the mechanisms of interference in immunoassays, there is no single procedure that can rule out all interferences (10). Prolactin tests generally use the sandwich immunoassay principle, but they differ in the technologies used for labeling. The Cobas 8000 system uses Electrochemiluminescence Immunoassay (ECLIA), where biotinylated and ruthenium-labeled prolactin-specific antibodies are used to form a sandwich complex UniCel DxI 800 system is a one-step sandwich chemiluminescent immunoassay using paramagnetic particles. It utilizes a polyclonal goat anti-PRL alkaline phosphatase conjugate and paramagnetic particles coated with a Mouse monoclonal anti-PRL antibody. The Alinity i 1000 system is a two-step automated test using Chemiluminescent Microparticle Immunoassay (CMIA) technology, where prolactin binds to anti-prolactin-coated microparticles, and an acridinium-labeled conjugate is added afterward. The Centaur XP Prolactin assay is a 2-site sandwich immunoassay using direct chemiluminescent technology. It involves two fixed antibodies: a goat polyclonal anti-prolactin antibody labeled with acridinium ester in the reagent, and a mouse monoclonal anti-prolactin antibody attached to paramagnetic particles in the Solid Phase. Several studies have identified alkaline phosphatase (ALP) as a potential source of interference in immunoassays (11-12). In a study by Herman et al., elevated ALP levels (>1000 U/L) were found to interfere with assays like DxIcTnI and hCG. Yıldız et al. reported a case of falsely low unconjugated estriol (uE3) levels in a 35-year-old pregnant woman due to assay interference. Initial screening on the UniCel DxI 800 analyzer indicated a high risk for Down syndrome, but re-testing on the IMMULITE 2000 XPi showed a normal result. Further investigations revealed that heterophile antibodies and alkaline phosphatase scavenger increased uE3 levels, confirming interference (12). The researchers stressed the importance of evaluating the effect of ALP interference during the method validation process, particularly in immunoassays that rely on ALP for signal amplification. In our study, adding Pool 3, which contains Scavenger ALP (a blocker related to ALP), did not eliminate the interference leading us to conclude that the interference was not related to ALP.

In conclusion, this case highlights the critical need for collaboration between laboratory professionals, clinicians, and assay manufacturers to identify and address assay interferences. Despite extensive investigations, the exact nature of the interfering agent could not be determined, emphasizing the challenges in resolving immunoassay interference. Ongoing education, research into underlying mechanisms, and improved strategies for managing interferences are essential for ensuring accurate testing and optimal patient care, particularly in conditions where hormone level fluctuations significantly impact treatment. Future efforts should focus on the identification of new interferences and developing strategies to minimize their impact on clinical practice, with manufacturers playing a key role in this process.

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