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Losing weight plus vitamin B12 following semaglutide administration? A case of false B12 deficiency

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Abstract

Semaglutide is increasingly used to lose weight. We describe a case of an obese woman with unremarkable medical history. Following semaglutide administration, she managed to lose 10 kg over a period of 8 months, but B12 levels were markedly decreased. She had a series of previous annual tests including B12 results during routine check-up, which were performed in the same laboratory of the university hospital of Ioannina in Greece and consistently found normal B12 levels. She is not vegetarian, she had no symptoms or signs related to macrocytosis, anemia or B12 deficiency, blood smear examination did not reveal macro-ovalocytes, tear-drop cells, polychromatophilia, or hyper-segmented neutrophils, and the reticulocyte, white blood cell and platelet counts were within the normal range. We additionally measured homocysteine and methylmalonic acid which were within the normal range, whereas the antibodies against intrinsic factor were also negative. B12 levels are commonly measured using competitive binding immunoenzymatic assay. We postulate that for assays employing antibodies, a possibility exists for interference by heterophile antibodies boosted by semaglutide. Importantly, in diabetic patients semaglutide follows the introduction of metformin, which is frequently associated with low B12 levels, and in those cases, we tend to attribute low B12 levels only to metformin.

Keywords: Semaglutide; B12; Obesity; Diabetes; GLP1; Case report.

Abbreviations: BMI: Body Mass Index, MMA: Methylmalonic Acid, HBT: Heterophilic Blocking Tube, GLP1-RA: Glucagon-Like Peptide-1 Receptor Agonist.

Case presentation

A 45-year-old, white woman had her annual routine laboratory exams, which showed vitamin B12 level of 96 pg/mL (Access vitamin B12 assay, Beckman Coulter). This is considered as severe B12 deficiency (normal range: 180-914, indeterminate range 145-180, deficiency <145 pg/mL) [1]. Eight months ago, she started semaglutide to lose weight. Her initial Body Mass Index (BMI) was 31. Semaglutide was gradually titrated to a dose of 1 mg weekly. She managed to lose 10 kg with a current BMI of 28. She had a series of previous annual tests including (and not limited to) B12 results during routine check-up, which were performed in the same laboratory of the university hospital of Ioannina in Greece and found normal B12 levels (Figure 1). Her medical history was unremarkable, and she was not on any other medication. She is a nonsmoker, does not consume alcohol, she is not vegetarian, has no animals and had no recent travel abroad. The woman is healthy, did not notice any changes of her physical condition, and no symptoms or signs related to anemia or B12 deficiency (including any neuropsychiatric abnormalities) were evident. Blood smear examination did not reveal any abnormal findings and in particular macro-ovalocytes or tear-drop cells, polychromatophilia, or hyper-segmented neutrophils. In the blood test results there was no macrocytosis with related anemia, and the reticulocyte, white blood cell and platelet counts were within the normal range. We repeated the same test in the same laboratory and again B12 levels were low. We additionally measured homocysteine and Methylmalonic Acid (MMA) which were within the normal range (not increased as expected in a case of B12 deficiency), and the antibodies against intrinsic factor were also negative. As she had previous normal B12 levels, and no symptoms or signs compatible with B12 deficiency, we finally repeated B12 measurement using an Heterophilic Blocking Tube (HBT, Scantibodies Lab), which contains a blocking reagent to inactivate heterophilic antibodies [2]. The repeated test reported more than 2-fold increase of B12 levels (250 pg/mL), although currently there is no reference range for B12 levels from the manufacturer.

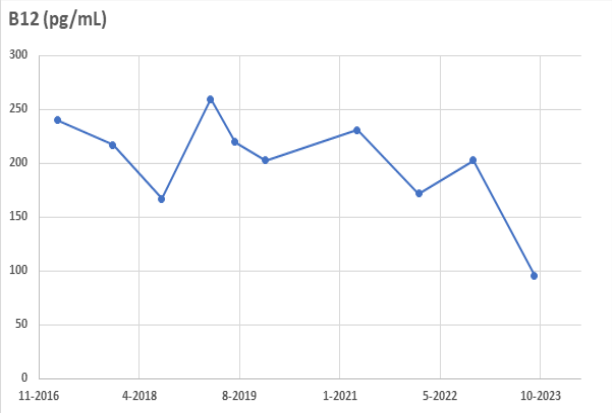

Figure 1: Trajectory of B12 levels as exported from the medical records of the University hospital of Ioannina.

Discussion

This is a case of false-low B12 levels following semaglutide administration. The Access vitamin B12 assay (Beckman Coulter) is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel along with alkaline potassium cyanide and dithiothreitol to converts all forms of vitamin B12 to the cyanocobalamin form. After neutralization, intrinsic factor-alkaline phosphatase conjugate and paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-intrinsic factor are added to the sample. Vitamin B12 in the sample binds to the intrinsic factor conjugate, preventing the conjugate from binding to the solid phase anti-intrinsic factor. The materials bound to the solid phase are held in a magnetic field and then the light generated by a reaction with a chemiluminescent substrate is measured with a luminometer. The light production is inversely proportional to the concentration of vitamin B12 in the sample [1]. For assays employing antibodies, the possibility exists for interference by heterophile antibodies particularly for patients regularly exposed to animals, or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments [1,3,4]. Competitive-binding assays are preferred over the other formats as they can more easily be automated for high-throughput analysis, as vitamin B12 is frequently requested. Testing for B12 during the evaluation of anemia or neuropsychiatric disorders, especially in an increasingly older population is constantly increasing. It is reported that of the vitamin B12 tests done in a laboratory serving 200,000 individuals, 8% were in the deficient range, 10% in the intermediate range and 42% in the normal range [5]. It is difficult to know how many of these may represent true B12 deficiency. False normal B12 levels have been reported in cases of megaloblastic pernicious anemia, because these competitive-binding assays are susceptible to significant interference due to the existence of anti-intrinsic factor antibodies. These false-normal vitamin B12 results are obtained in 22-35% of patients with confirmed pernicious anemia [6]. Conversely, in our case we found false-low vitamin B12 levels. In cases of non-anticipated low or normal B12 levels, where a discordance between the signs and symptoms of an individual and B12 levels exists, it is useful to measure plasma total homocysteine and MMA levels, which are markedly elevated in over 95% of patients with true vitamin B12 deficiency. Additionally, holo-Transcobalamin (holoTC), which represents the active fraction of plasma cobalamin, might be more specific for vitamin B12 deficiency [7,9]. Although previous publications consider holoTC as the best predictor of low vitamin B12 status, with Area Under the Curve (AUC) of 0.9, which is significantly better than the next best predictors serum total vitamin B12 (AUC=0.8) and MMA (AUC=0.78) [10], the frequently used holoTC cut-off values of 32-35 pM result in a diagnostic sensitivity of only 75% to 80% and a diagnostic specificity of only 55% and 60% [11,13]. In this case, we additionally used Heterophilic Blocking Tube assay, which contains a blocking reagent composed of specific binders which inactivate heterophilic antibodies. Once the specific binders have bound to the heterophilic antibodies, the antibodies are no longer able to cause immunoassay interference. False positive heterophilic interference in plasma or serum for immunoassays have been mainly reported for hormones, ferritin, hepatitis B surface antigen, creatine phosphokinase-MB, and cancer markers [1]. The manufacturer recommends that the HBT is used for a secondary confirmation assay, when the results from the first assay (initial sample not treated with HBT) are questionable. If the assay result from the HBT treated sample is different from that of the untreated sample, the difference is due to heterophilic interference [2]. In this case, since there is no official reference range for B12 levels when HBT is used, we report only the mark

difference of B12 levels between these 2 assays. It is the first time that a Glucagon-Like Peptide-1 Receptor Agonist (GLP1-RA), semaglutide, is associated with false-low B12 levels. This is an important finding for 2 reasons: first, GLP1-RAs follow the introduction of metformin in diabetic patients. Metformin is frequently associated with low B12 levels [14], and in those cases we tend to attribute low B12 levels to metformin. The risk of low vitamin B12 levels increases with higher metformin dose, longer treatment duration, and in patients with risk factors for vitamin B12 deficiency [15]. Thus, we administer B12 without any further investigation. Secondly, semaglutide is increasingly used for weight reduction in non-diabetic individuals (without metformin); thus, it is important to know if low B12 levels represent a true B12 deficiency and whether further investigation is needed with holoTC, homocysteine, and MMA (or even HTB).

Conclusion

Low B12 levels in a healthy individual on semaglutide trying to lose weight should be meticulously checked according to previous serial B12 measurements and investigated with additional tests (holoTC, homocysteine, MMA) before we reach to a definite diagnosis of B12 deficiency and begin B12 replacement therapy.

Declarations

Conflict of interest: None related to this work.

Statement of ethics: This retrospective review of patient data did not require ethical approval in accordance with local/national guidelines. Written informed consent was obtained from the patient for publication of the details of the medical case and any accompanying images.

Authors contribution: EER, EEN: Concept and design, data acquisition, data analysis, literature review, manuscript writing, manuscript review; EJ, EN, VT, GK, GA: literature review, manuscript writing, manuscript review. All authors agreed to proceed to publication.

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