Vitamin B₁₂ Immunoassay on Roche Elecsys 2010: Effects of High Excess Concentration of Serum Vitamin B₁₂ in CKD Patients on Parenteral Administration

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Abstract Vitamin B₁₂ being water soluble is excreted in the urine when administered in excess. The probability of finding an abnormally excess serum concentration would be almost surreal. We report a peculiar clinical situation that may impact the vitamin B₁₂ immunoassay on the Roche Elecsys 2010 due to excess analyte concentration. In separate episodes (Feb and June 2010), the Biochemistry laboratory of a tertiary-care hospital, Kolkata, India, encountered two critically ill patients with background chronic kidney disease (CKD), low urine output, and on cyanocobalamin supplementation, who had serum vitamin B₁₂ concentrations far exceeding expected values; even post dialysis. The B₁₂ assays (pmol/l) were performed using electrochemiluminescence immunoassay on Roche Elecsys 2010, the assay validity confirmed by concomitant quality control runs. The immunoassays failed to deliver results, flagged with “*signal level below limit*”. Biotin therapy was ruled out as a possible interferent. In the first episode, re-assay of a repeat draw yielded same outcome; outsourcing on Immulite provided concentration of >738 pmol/l. Serial dilution gave result of >29520 pmol/l on Elecsys 2010. In the second, we gained from past experience. Vitamin B₁₂ concentration >59040 pmol/l was conveyed to the treating nephrologist the very day. The B₁₂ immunoassay on the Elecsys 2010 employs sequential incubation steps for competitive binding that is compromised in the event of abnormally excess B₁₂ concentration in patient sera akin to the prozone effect. This knowledge may be beneficial while assaying sera of CKD patients to

avoid financial loss due unnecessary repeats and delay in turnaround time.

Keywords Vitamin B₁₂ immunoassay · Roche Elecsys 2010 · Interference · Renal failure · Hook effect · Dialysis

Abbreviations

ICU Intensive care unit
CKD Chronic kidney disease

Introduction

Water soluble vitamins such as the B complex group or vitamin C are excreted in the urine if administered in excess. High circulating levels of these supplements are therefore unlikely.

In hospitalized patients, vitamin B₁₂, a water soluble vitamin, is prescribed in high doses to patients with renal failure, alcoholism, neurological deficits, who are predisposed to macrocytic and nutritional anemias [1].

The hospital laboratory is often frequented with requests for serum Vitamin B₁₂ assay. In an unexpected scenario, we report below cases of vitamin B₁₂ assay in patients of chronic kidney disease (CKD) receiving high doses of vitamin B₁₂, where high circulating concentration of the analyte led to failure of the assay technique to generate valid results.

Case Reports

In two similar scenarios, a 64-year-old and a 72-year-old gentleman were admitted to the medical-surgical intensive

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care unit (ICU) of our tertiary-care hospital in Feb 2010 and June 2010, respectively who had their vitamin B₁₂ assayed in the laboratory.

In the first case, the patient had complains of fever, cough and shortness of breath. With other co-morbidities such as diabetes mellitus, hypertension, coronary artery disease, and chronic obstructive pulmonary disease, he also had background alcoholism and peripheral neuropathy for which he was prescribed high doses of vitamin B₁₂. The patient was a known case of CKD and was on occasional haemodialysis. On admission, serum urea and serum creatinine were detected at a level of 86.39 mmol/l (242 mg/dl) and 488.83 μ mol/l (6.41 mg/dl), respectively along with low urine output thereby indicating superimposed acute renal failure, for which the patient was on a daily dialysis regimen. Among with other specific medications, the patient was receiving injection optineuron (contains thiamine HCl, pyridoxine HCl, riboflavin sodium phosphate, cyanocobalamin, nicotinamide), equivalent to 3,000 mcg of cyanocobalamin per day.

The second case was a patient of suspected lower respiratory tract infection, on ventilation due to worsening drowsiness and hypotensive requiring vasopressor support. He was a known case of CKD V on regular dialysis. His admission serum urea level was 14.28 mmol/l (40 mg/dl) and serum creatinine was 220.39 μ mol/l (2.89 mg/dl). From the third day of admission, the patient was being supplemented with injection optineuron, equivalent to 1,000 mcg of cyanocobalamin per day.

On receiving request for the patient's serum vitamin B₁₂ quantification, we tested the sera using Roche Elecsys 2010 immunoassay analyzer that employs the test principle of competitive electrochemiluminescence immunoassay. The assay has a linearity range of 22.1–1,476 pmol/l. The assay involves binding of vitamin B₁₂ in the sample to the ruthenium labeled-intrinsic factor complex. Sequentially, biotin labeled vitamin B₁₂ is added to the reaction that competes for the leftover vacant binding sites on the intrinsic factor. Streptavidin coated microparticles (solid phase), binds the biotin labeled vitamin B₁₂ and ruthenium labeled-intrinsic factor complex. All the unbound substances are washed away leaving the solid phase magnetic microparticles bound with vitamin B₁₂-biotin and ruthenium labeled IF complex, which generates chemiluminescent emission signal that is inversely proportional to the concentration of vitamin B₁₂ present in the patient sample.

In this particular assay of the first patient sample, no result was generated, indicated by a flag of “*signal level below limit*”. We initially suspected gross interference in the sample and suggested repeat phlebotomy. The repeat sample also revealed a similar consequence. A thorough patient history from the treating consultant did not explain the occurrences including absence of a possible biotin

therapy that otherwise may have interfered with the assay. In an effort to rule out unspecified assay interferences on the Roche Elecsys 2010 system, we crosschecked the serum sample to a referral laboratory on the Immulite 2000, Siemens, which readily gave a result of >738 pmol/l, thereby indicating analytical sensitivity error in our assay system for a sample with high concentration of vitamin B₁₂. From the –20°C aliquot of the primary sample, we ran a fivefold dilution test with a result of >7,380 pmol/l, followed by repeat on a 20-fold dilution that yielded a value of >29,520 pmol/l. The report was finally communicated to the treating physician; 48 h after receipt of the requisition.

For the B₁₂ assay of the second patient, we found no valid result, which was also flagged as “*signal level below limit*”. We ruled out biotin therapy that could have been a possible interferent. Gaining from past experience, we repeated the assay after several folds of serial dilution of the patient serum. Concentrations greater than 59,040 pmol/l of vitamin B₁₂ were obtained. This result was conveyed to the treating nephrologist on the same day.

Discussion

So, why did the assays fail to generate valid results? Surely, both patient sera had excess amounts of vitamin B₁₂ given the fact that they were on high doses of supplementation with compromised urinary output. Though the patients were on frequent hemodialysis, the excess vitamin B₁₂ concentration in the sera could be explained from the findings of Ferhman-Eckholm et al. where serum cyanocobalamin levels have been shown to remain unaltered even after hemodialysis [2].

We deduced that in the Roche Elecsys 2010 system, the abnormally excessive patient sera analyte had consumed all available sites on the intrinsic factor (IF) in the first incubation step. Sequentially, in the next incubation step when biotin labeled vitamin B₁₂ was added, there were no vacant sites on the IF for it to bind. Thus no signal was generated. This is akin to the hook or the prozone effect where excess antigen in the serum of the patient saturates the capture and/or signal antibodies either preventing signal generation or leading to falsely low levels of the antigen being measured, although only seen in two step immunoassays [3, 4]. On the contrary, the test principle of the Immulite system employs a single incubation step where the patient analyte, labeled hog intrinsic factor are simultaneously added on the vitamin B₁₂ analog coated on polystyrene bead allowing for competitive binding to occur and hence for a possible signal generation in spite of excess analyte concentration.

In conclusion, the laboratory biochemist needs to be aware of such a phenomenon in the Roche Elecsys B₁₂

assay for unexpected cases such as patients of renal failure receiving high doses of the vitamin, because it could lead to unnecessary retesting and expenditure and delayed reporting that may decrease clinician's confidence in laboratory testing. In cases of patients with advanced renal failure, it is important to perform dilution curves if results do not match with clinical presentations or, for assays that fail to yield results due to interference in signal generation. To our knowledge, this is the first reported case of a phenomenon akin to hook effect for vitamin B₁₂ immunoassay in patients with advanced renal failure requiring dialysis. Being aware of this phenomenon will help laboratory staff to manage such assays with minimum retesting and in preventing delayed turnaround time.

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