Case Report

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Alkaline phosphatase ınterference in an unconjugated estriol assay causing a false positive Down syndrome screening result

[Yanlış Pozitif Down Sendrom Tarama Sonucuna Sebep Olan Unkonjuge Estriol Testindeki Alkalen Fosfataz İnterferansı]

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Abstract

Objective: Decreased unconjugated estriol (uE3) concentrations increase calculated risk of Down syndrome. Therefore, falsely low uE3, due to assay interference, have the potential to cause false-positive screening results. Here we present a 35 years old woman with a pregnancy of 17 + 2 weeks.
Materials and methods: Second-trimester screening test was performed on the UniCelDxI 800 (Beckman Coulter, Brea, CA, USA) analyzer and her uE3 level was 0.21 ng/mL (0.21 MoM). Risk calculated for DS was 1/8. Measurements were repeated on IMMULITE 2000 XPi (Siemens Healthcare Diagnostics Inc., USA). uE3 result was 0.614 ng/mL (0.97 MoM). The risk for DS was negative with this system. There was no sign of fetal anomaly on three-dimensional ultrasound examination and cell-free fetal DNA screening test. We suspected assay interference for uE3.
Results: Serial dilutions of serum samples revealed nonlinearity. 36.3% increase was observed with heterophile antibody blocking tubes. The post-polyethylene glycol treatment resulted approximately the same uE3 levels as IMMULITE system. Addition of alkaline phosphatase Scavenger to serum increased the result by 90% showing that falsely low E3 result was due to an interferent reacting on assay medium.
Conclusion: Laboratories should be aware that falsely low uE3 results due to interference may be obtained.

Keywords: Alkaline phosphatase; Unconjugated estriol; False positive; Interference.

Öz

Amaç: Azalmış unkonjuge estriol (uE3) düzeyleri Down sendrom riskini arttırır. Bu yüzden, ölçüm interferansına bağlı yanlış düşük uE3 düzeyleri yanlış pozitif tarama sonuçlarının bulunmasına yol açar. Bu çalışmada 17 + 2 haftalık gebeliği olan 35 yaşındaki bir kadın olgu sunuldu.
Gereç ve Yöntem: İkinci trimester tarama testi UniCel-DxI 800 (Beckman Coulter, Brea, CA, USA) analizöründe ölçüldü ve uE3 düzeyi 0.21 ng/mL (0.21 MoM) bulundu. Down sendrom riski 1/8 olarak hesaplandı. Ölçümler IMMULITE 2000 XPi (Siemens Healthcare Diagnostics Inc., USA) analizöründe tekrarlandı. uE3 sonucu 0.614 ng/mL (0.97 MoM) bulundu. Down sendrom riski bu sistemde negatiftir. Üç boyutlu ultrason incelemesinde ve cell-free fetal DNA tarama testinde fetal anomali bulgusuna rastlanılmadı.
Bulgular: Örneklerin seri dilusyonunda nonlinearite gözlendi. Heterofil antikor blokan tüpler ile %36.3 artış

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gözlendi. Polietilen glikol uygulama sonrası İmmulite sistemdeki sonuçlara benzer uE3 sonuçları saptandı. Seruma alkalen fosfataz scavenger eklenmesi sonrasında uE3 sonucunda %90 artış gözlendi. Yanlış düşük uE3 sonucunun reaksiyon ortamında bulunan interferana bağlı olduğu düşünüldü.

Sonuç: Laboratuvar çalışanları ölçüm interferansına bağlı yanlış düşük uE3 düzeylerinin görülebileceğinin farkında olmalıdır.

Anahtar kelimeler: Alkalen fosfataz; Unkonjuge estriol; Yanlış pozitiflik; Interferans.

Introduction

uE3 is measured, as a part of second-trimester DS screening tests and analysis of uE3 is always performed by immunoassay technique [1]. Interferents such as heterophile antibodies, human anti-animal antibodies, autoanalyte antibodies, rheumatoid factors (RFs) and other proteins in a patient sample, cross-react with the antibodies used [2] or assay detection components, such as streptavidin or ALP labels of reagents. In enzyme-labeled immunoassays, the presence of inhibitors or activators of the detection enzyme may alter the signal and thereby the immunoassay results. This interference in immunoassays can be more difficult to identify in the setting of prenatal screening programs [3], where laboratory testing is applied to identify those at risk of a disorder, in a population who have not sought medical attention on account of symptoms of that disorder [4].

In this case report, we investigated the interference causing low uE3 on UniCelDxI 800 immunoassay system.

Patients and methods

We described a 35 years old pregnant woman who has a pregnancy of 17 + 2 weeks. DS and SLOS (Smith-Lemli-Opitz Syndrome) risk was positive. It was her fifth pregnancy and she had a history of two abortuses at 4th and 6th weeks and she had no history of a child with an anomaly, consanguinity marriage, and medication.

uE3, human chorionic gonadotropin (HCG), and alfa-fetoprotein (AFP) measurements were done on the UniCelDxI 800 (Beckman Coulter, Brea, CA, USA) analyzer and their corresponding catalog numbers were: 33570, 33210 and 33570, respectively). The Access uE3, HCG and AFP are competitive binding immunoenzymatic assays. HCG, AFP, and uE3 tests were repeated in another laboratory (Acibadem University School of Medicine Laboratory) working with a different system IMMULİTE 2000 XPi (Siemens Healthcare Diagnostics Inc., USA). Corresponding catalog numbers were: L2KAP2, L2KCG2, and L2KUE32, respectively. The technique is a solid-phase, enzyme-amplified chemiluminescence immunoassay. We performed serial dilutions (1/2, 1/4 and 1/8) using the manufacturer's zero calibrator for both our patient's serum and a control sample. For heterophilic antibody investigation, heterophilic blocking tube (HBT) (Scantibodies Laboratory, Inc. USA, cat. no: 3IX762) was used for a secondary confirmation assay and compared with the original results. The HBT contains a unique blocking reagent composed of specific binders which inactivate heterophilic antibodies. Each tube contains enough reagents to inactivate the heterophilic antibodies in 500 µL of sample. The reagent is in the form of a lyophilized pellet at the bottom of the tube. The serum of the patient was then subjected to precipitation with PEG 6000 (product no. 29577; Merck Ltd.) to remove interfering antibodies [5]. For further investigation, the patient sample was sent to Beckman Coulter complaint handling unit laboratory and the two different blocking reagents were applied to the patient's serum each in duplicate and were run through the immunoassay. Firstly, Pool 1 was composed of different blockers (PolyMak 33 and HBR-1), which are animal derived antibodies. PolyMAK-33 was a polymerized murine IgG1 preparation, superior to polyclonal Mouse immunoglobulins in blocking heterophilic antibody activity [6]. Heterophilic Blocking Reagent (HBR-1; Scantibodies) contains immunoglobulins of murine origin with specific binders that neutralize by active attachment to the heterophilic antibody. The second blocking reagent was Scavenger ALP. A percentage difference of ±25% was expected if no interference was present.

Results

Detection limit of uE3 was 0.017 ng/mL and total coefficient of variation (CV) was 10.5% and 3.442% for concentrations of 0.267 and 4.83 ng/mL, respectively. Total CV was 9.8% and 6.52% for concentrations of 0.98 and 3.17 ng/mL, respectively and acceptable. Randox International Quality Assessment Scheme external quality assessment maternal screening program (Laboratory code 128524/A) was used for accuracy and standart deviation index (SDI) for the relevant date was (−0.80 SDI). Her uE3 level was 0.21 ng/mL (0.21 MoM). uE3 measurement was repeated by the same assay and the result was 0.247 ng/mL, still inappropriately low. Her HCG level was 101,876 mIU/mL

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(3.55 MoM) and AFP level was 44.1 ng/mL (1.23 MoM). Risk calculated by the software (Benetech PRA Ver. 2.4.1.1. (Benetech Inc., Toronto, Canada) for DS was 1:8 and far above the negative cut-off of 1/200. Also, SLOS risk was positive (Figure 1). Three-dimensional ultrasound examination by an expert gynecologist was performed and there was no sign of fetal anomaly. Cell-free fetal DNA screening test was also performed in Natera Inc, San Carlos California. Monosomy X, trisomy 21, 18 and 13 estimated panorama risk scores were <1/10,000 and these diseases were excluded. The low uE3 value was inconsistent with results expected from a normal pregnancy and we attempted to investigate the cause of this interference by proceeding studies. Pregnant's serum ALP value was 245 IU/L and second trimester reference range was 25–126 IU/L for our method. DS risk was also calculated using Siemens PRISCA Prenatal Risk Calculation Software v.4.0. The risk for DS was negative and calculated as 1:321 (Figure 2). Linearity was observed for the control sample while our patient's serum revealed nonlinearity and higher uE3 results suggesting assay interference. For investigation of heterophilic antibody, analysis were carried out simultaneously on a control sample and our patient's serum. Percent difference was found as 8.9% and acceptable for the control patient while uE3 level of our patient was increased by 36.3% after HBT. We thought the presence of any kind of antibodies reacting partially with anti-animal antibodies present in HBT. We performed a RF measurement on IMMAGE 800 System (Beckman Coulter, Brea, CA, USA) with a nephelometric method and it was found in reference range 10.8 IU/mL (0–15) Also, serum immunofixation analyses revealed no monoclonal peak. Analyses were carried out simultaneously on a control sample and our patient's serum and PEG recoveries were determined. PEG recovery was 93% and not significant for the control sample. Our patient's result was found as 0.588 ng/mL; with a recovery of 283%. This result was approximately the same as the result obtained on Siemens Immulite

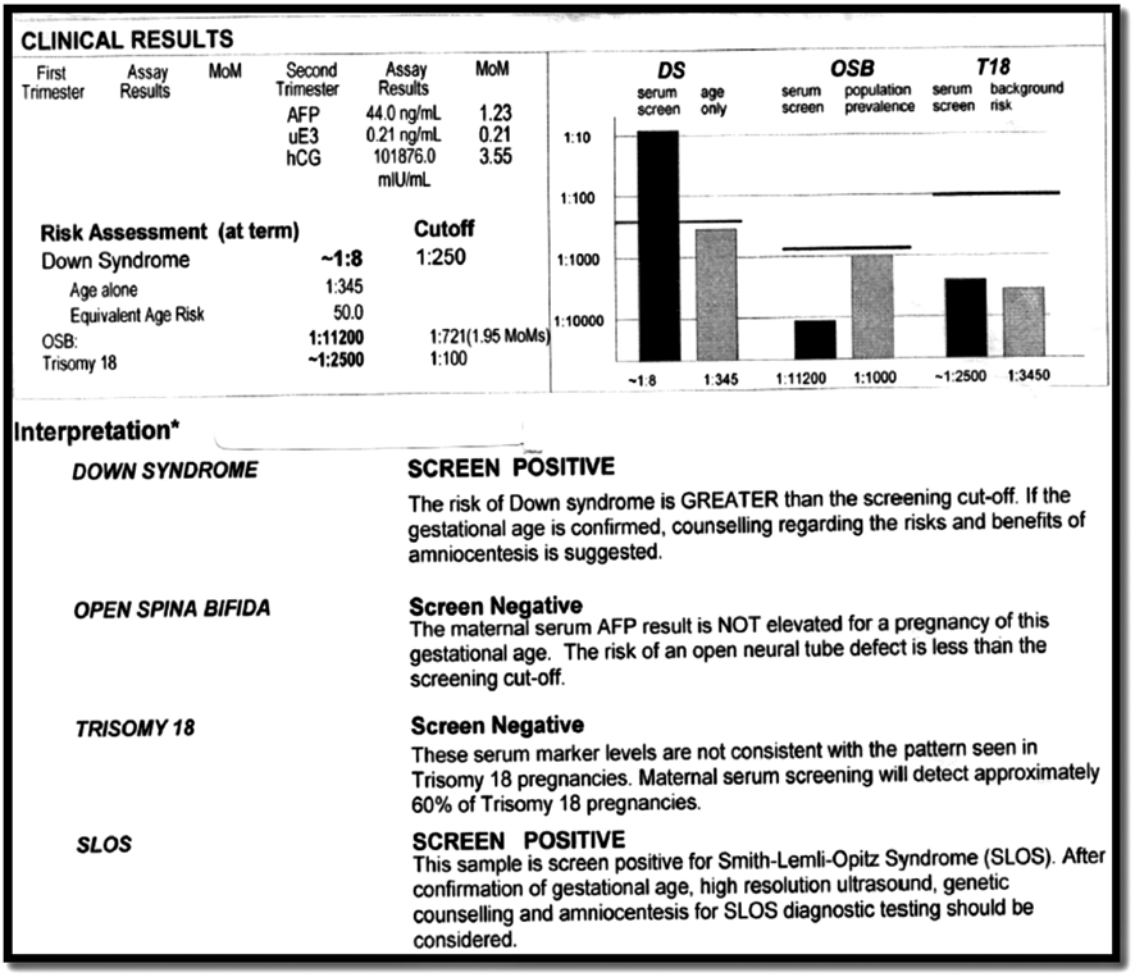


Figure 1: Second-trimester screening results analyzed by UniCelDxI 800 System.

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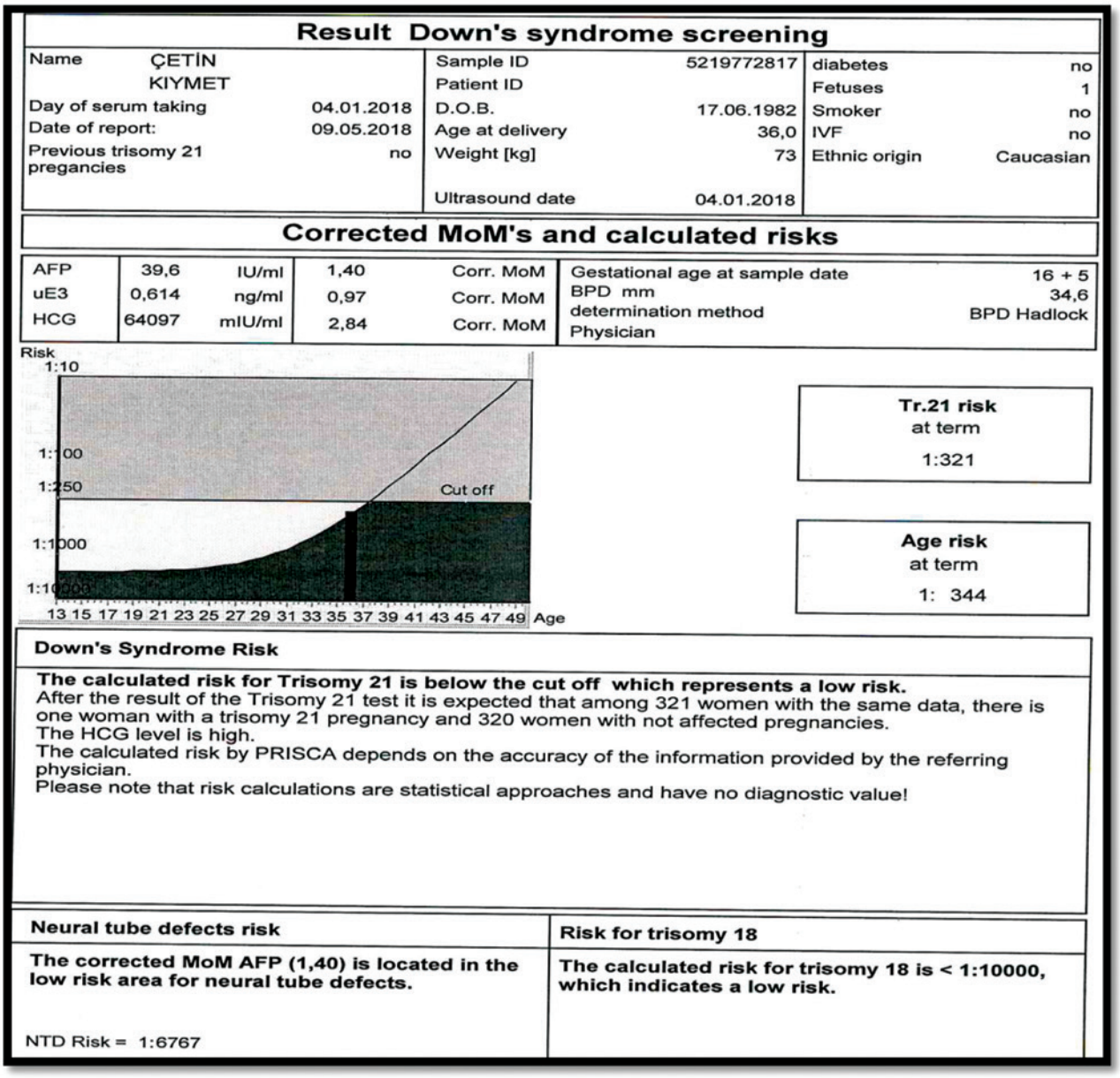


Figure 2: Second-trimester screening results analyzed by IMMULITE 2000 XPi System.

system. We observed that PEG treatment succeeded in the elimination of interferent [5]. Percentage differences after the addition of Pool 1 were 11% and 14%. Percentage differences after the addition of scavenger ALP were 91% and 80% indicating a successful blockage.

Discussion

To the best of our knowledge, this is the first case with interference in uE3 assay with high ALP activity in a pregnant woman.

In our case, exact nature of the interference was not identified. Interference might be due to increased ALP probably with a different origin and/or molecular form (an isoform with a protein moiety bound). It might bind to conjugate in reaction medium and/or even bind to leucocyte membranes in the patient sera. In all of these situations it might act as a macromolecule and thus get trapped in the reaction mixture while washing or cause an interference just like an antibody. It could get trapped partially by HBT (because non-specific) and could be precipitated with PEG. Second, human anti-ALP antibodies might be present in samples and bind to the conjugates, the

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endogenous ALP and also to the paramagnetic particles or get trapped in the pellet during washing. What we are sure of is that there is an interferent in pregnant's sera that was blocked with Scav-ALP and this interference did not occur in another system using different antibodies and washing system. Earlier generations of some of these ALP-labeled immunoassays were susceptible to endogenous ALP interference [7, 8] so improved washing procedures were incorporated into contemporary versions. In all of the scenarios signal production increased causing false negative uE3 result. Scav ALP was inactived native ALP produced from calf intestine and used as blocking reagent in immunoassays where native ALP is used as conjugate. This inactive calf ALP together with a protein matrix offered a similar medium to interfere. The interferent was blocked with this scavenger and no additional signal occurs in the reaction medium because it is inactive.

There are a few studies about ALP labeled immunoassay interference. In Herman study, elevated ALP (>1000 U/L) was thought as the interfering agent with DxIcTnI and hCG assays [9]. They recommended to evaluate the magnitude of ALP interference in method validation of all sensitive immunoassays that use ALP for signal amplification. In another study with the Dade Stratus cTnI fluorometric enzyme immunoassay, cTnI was affected by high concentrations of ALP [8]. In the same study, no interference by high concentrations of ALP were found for the microparticle immunoassay assay (Abbott Laboratories) or the chemiluminescent assay (Bayer Diagnostics). cTnı level was found falsely elevated with Access AccuTnI Assay which also used ALP. After addition of HBR-1, cTnı result reduced by greater than 90% indicating succesful blockage of patient's heterophilic antibodies. There was no information about ALP value of the patient, probably normal [10].

Conclusion

There is no single procedure to rule out all interferences as there is no way to predict assay interference a priori. A close communication between clinical and laboratory staff is necessary to avoid unnecessary investigations and inappropriate treatments.

Ethical considerations The study was approved by the Ethics Review Committee of Dr. Lütfi Kırdar Kartal Research and Training Hospital. (2018/514/130/7 29.05.18).
Conflict of interest: Authors declare that there is no conflict of interest.

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