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Cell-free DNA testing: is it reliable? A case report

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Summary

In this article, the authors reported the findings of a false negative case of cell-free DNA (cfDNA) testing for trisomy 21. The cfDNA test was performed due to the increased nuchal translucency during the first trimester scan. After receiving the "normal" result of the test, the patient was followed up. However, intrauterine growth retardation and ventriculomegaly were detected in the second trimester of pregnancy and fetal karyotyping revealed trisomy 21.

Key words: False negative cell-free DNA testing; Fetal aneuploidy; Prenatal diagnosis; Trisomy 21.

Introduction

Several screening tests and ultrasonographic markers are used to isolate high-risk pregnancies for fetal aneuploidy from low-risk group [1]. Today, cell-free DNA (cfDNA) based non-invasive testing is being used with an increasing demand. It has a detection rate which is greater than 99% for trisomy 21, with a false positive rate of less than 0.1% [2]. Since recent studies demonstrated false-positive and false-negative test results, clinicians need to be aware of these rates of this screening test [3].

Case Report

A 35 -year-old woman, gravida 3, para 2, was referred to the present antenatal clinic at 12 weeks of gestation because of increased nuchal translucency. Ultrasonographic examination revealed a 12 weeks and 3 days gestation with a crown-rump length (CRL) measuring 54 mm and nuchal translucency 4.8 mm in thickness. Nasal bone was present, no reverse "a" wave was observed on ductus venosus Doppler evaluation. The family was offered invasive diagnostic procedure, namely chorion villus sampling (CVS) and also informed about cfDNA-based non-invasive testing. The patient refused CVS because of the miscarriage risk related to CVS and preferred cfDNA. The result was reported as "normal" that was contemplated with a fetal fraction rate of 9.98%. The patient was followed up in a private hospital until 22 weeks of gestation, but then was referred to the present clinic again because of the presence of ventriculomegaly. In detailed ultrasound evaluation, head circumference, biparietal diameter, and abdominal circumference were concordant with 20+ weeks, but fetal long bones were consistent with 18+ weeks of gestation. Both atria of the lateral ventricles were dilated, measuring 15.99 mm (severe ventriculomegaly, Figure 1). Flattened nasal bridge was also detected. The fetus was externally female. The patient was reoffered invasive test for fetal karyotyping and cordocentesis revealed 47, XX,+21. The pregnancy was terminated upon the family's request. However, cytogenetic analysis of both the fetus and the placenta could not be performed because of the family's refusal of further investigation.

Discussion

Cell free DNA testing is being used with increasing demand since 2011. Although in singleton pregnancies, for trisomy 21, the detection rate of cfDNA is reported as 99.2% with a false positive rate of 0.09%; the prevalence of false negative cell free DNA testing is not clearly known [2, 4, 5]. The false results of the test may be explained by patient and pregnancy related factors such as maternal obesity, malignancy, co-twin demise, confined placental mosaicism. and technical limitations of the test. In the present case, the patient was not obese with a body mass index of 28.3. The yielded cfDNA fraction was 9.98%, a rate above the acceptable satisfactory level of 3-4% [6].

The most important aspect to be known regarding cfDNA testing is that the circulating cfDNA in maternal plasma derives from the cytotrophoblasts of the chorionic villi, not the fetus. cfDNA testing reflects the genetic status of the cytotrophoblasts, not the actual fetal karyotype. The main problem in the present case was that the authors did not analyze the genetic status of the placenta. In this case, cfDNA testing was reported as "normal", indicating that the cytotrophoblasts were karyotypically normal. However, cordocentesis revealed trisomy 21, indicating that the fetus itself did not possess normal karyotype. Although the placenta and the fetal tissue could not be analyzed postnatally in this case, the authors considered that the most likely explanation was chromosomal aberration confined only to the fetus that was not present in the placenta. In the case of a

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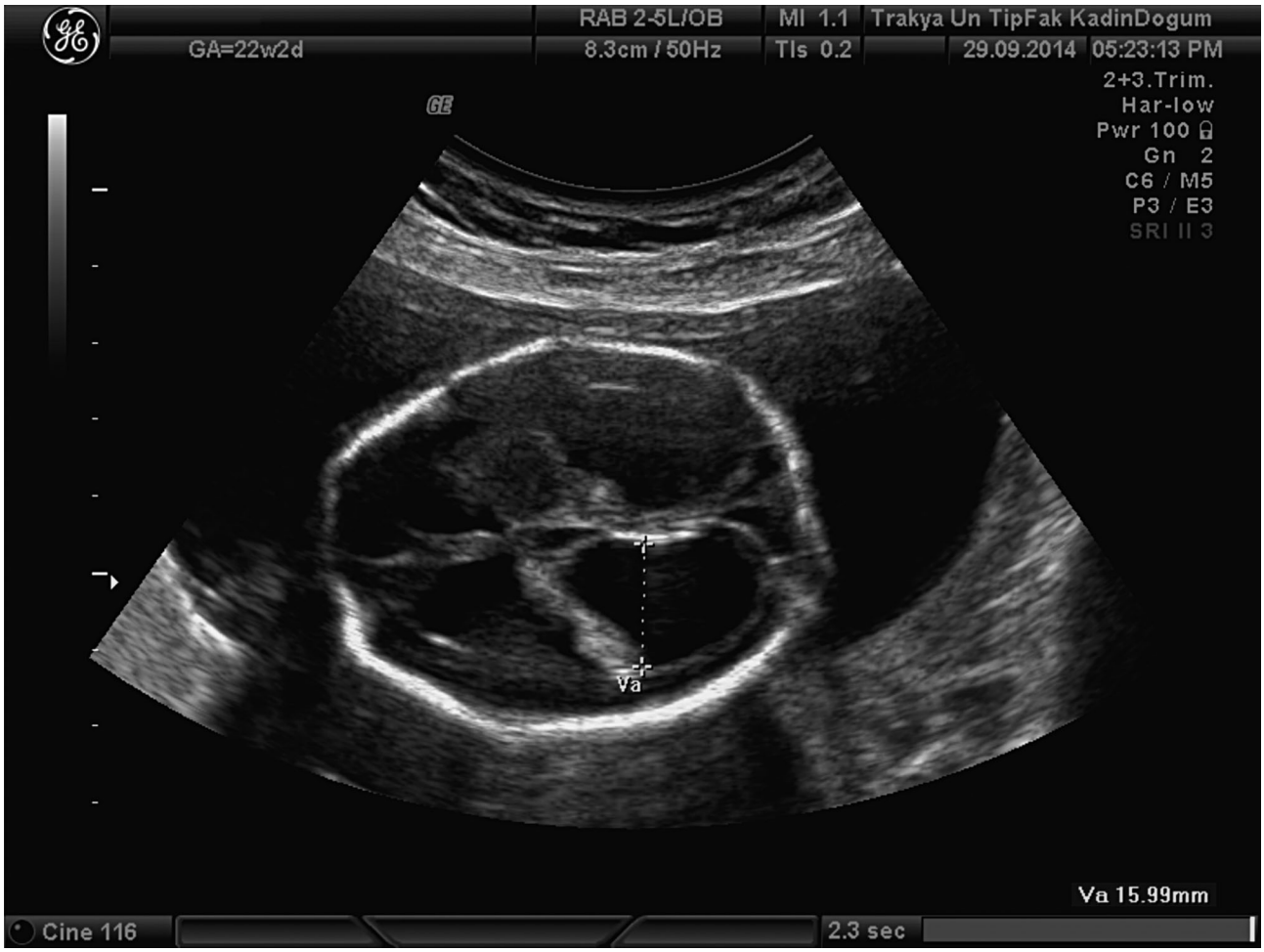

Figure 1. — Ultrasound showing severe ventriculomegaly.

normal karyotype in the cytotrophoblast with abnormal karyotype in the fetus, two forms of mosaicism should be considered. The first is the presence of abnormal chromosomal cell lineage in both the fetus and the mesenchymal core, with normal karyotype in the cytotrophoblast. The second is called confined fetal mosaicism, which is extremely rare. In this form, cytotrophoblasts and the mesenchymal core have normal karyotype, whereas the fetus is only affected. These two forms of mosaicism could potentially be missed from the cfDNA testing. On the other hand, cfDNA testing evaluates only the DNA of the cytotrophoblasts. It can be interpreted that the results of the cfDNA testing will never reach those of diagnostic invasive tests because its genetic analysis is limited only to the cytotrophoblasts. For instance in CVS, cytogenetics of chorionic villi are studied by two techniques: short- and long-term cultures. Cells that are cultured in short-term originate from the cytotrophoblasts, but cells of the long-term preparation originate from the mesenchymal core, another cell population which together with the fetus develops from the inner cell mass. Thus, the results of the cfDNA testing are nearly equal to those obtained from short-term culture. It is also noteworthy to mention that different karyotypes could be present in different compartments (cytotrophoblasts, mesenchymal core, and the fetus) leading to false results [3].

Although in the present patient the result of cfDNA was reported as “normal”, second trimester ultrasonographic findings, i.e. early growth retardation and severe ventriculomegaly, were suspicious for trisomy and led the authors to reinvestigate for fetal aneuploidy. Cordocentesis resulted as trisomy 21. Thus, the importance of the second level ultrasound should not be overlooked by a negative cfDNA test. Also, cfDNA should not be used as a substitute for diagnostic testing. Enlarged nuchal translucency is an obvious anomaly and the patient should be offered genetic counseling and diagnostic testing, together with detailed anatomic evaluation in the second trimester. The present case highlights that in the presence of structural abnormality that is strongly suggestive of a particular aneuploidy,

one of the invasive diagnostic procedures should be performed directly without wasting time. During pre-test counseling with the future parents, they should be informed about cfDNA testing that it is solely a "screening" test. In the case of positive test result, the finding should be confirmed by one of the diagnostic invasive procedures [7]. However, as shown in the present case, one should not rely on a negative cfDNA testing in the presence of strong ultrasound markers like increased nuchal translucency.

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