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Prenatal diagnosis of Down syndrome in dizygotic twin pregnancy

Prognoza prenatalna zespołu Downa w ciąży bliźniaczej dizygotycznej

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Abstract

We present a case of a 33-year-old pregnant woman who had a transvaginal ultrasound performed at week 9 of gestation. A dichorionic diamniotic twin pregnancy, with symmetrically developing fetuses, was confirmed.

Chromosomal defect markers (NT, NB, DV, TV) were analyzed in the first genetic test, performed according to the Fetal Medicine Foundation (FMF) criteria, and the double marker test was performed (PAPP-A protein and free β-hCG concentrations in patient serum were determined).

In the subsequent diagnostic procedures, the patient was offered and consented to amniopuncture after week 15 of gestation. The material obtained in the course of that invasive procedure allowed to identify a normal male karyotype – 46, XY in the first fetus (Fetus I). Cytogenetic analysis of the material from the second fetus (Fetus II) resulted in the diagnosis of an abnormal female karyotype – 47, XX, +21.

Based on the analyzed clinical case, we present the difficulties of performing prenatal diagnosis in a dizygotic twin pregnancy. The results prove the applicability and efficacy of prenatal diagnostics tests based on the FMF criteria also in twin pregnancies.

Key words: **pregnancy** / **twin pregnancy** / **Down syndrome** / **PAPP-A protein** /

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Streszczenie

Przedstawiono opis przypadku 33 letniej ciężarnej, u której w 9 tygodniu ciąży wykonano USG z wykorzystaniem sondy przezpochwowej i potwierdzono ciążę bliźniaczą dwuowodniową dwukosmówkową z symetrycznie rozwijającymi się płodami.

W pierwszym badaniu genetycznym wykonanym zgodnie z kryteriami FMF dokonano analizy markerów defektów chromosomalnych tzn.: NT, NB, DV, TV oraz wykonano test podwójny (oznaczono stężenia w surowicy ciężarnej białko PAPP-A i wolną β-hCG).

W kontynuacji postępowania diagnostycznego ciężarnej zaproponowano wykonanie amniopunkcji po 15 tygodniu ciąży, na co wyraziła zgodę. Uzyskany w przebiegu procedury inwazyjnej materiał i późniejsza jego analiza pozwoliła rozpoznać u płodu pierwszego (Płód I) prawidłowy kariotyp męski – 46, XY. Cytogenetyczna analiza materiału płodu II (Płód II) dała podstawy do rozpoznania nieprawidłowego kariotypu żeńskiego – 47, XX, +21.

Na podstawie analizowanego przypadku klinicznego przedstawiamy trudności realizacji diagnostyki prenatalnej w ciąży bliźniaczej dizygotycznej. Przedstawione wyniki wskazują na skuteczność diagnostyki prenatalnej opartej na kryteriach FMF także w odniesieniu do ciąży bliźniaczej.

Słowa kluczowe: **ciąża** / **ciąża bliźniacza** / **zespół Downa** / **białko PAPP-A** /

Introduction

Down syndrome (formerly mongolism) belongs to the most common genetic abnormalities caused by the presence of all or part of an extra 21st chromosome. Down syndrome was first described by a British physician, John Langdon Down, in 1866 [1]. Mechanisms which include chromosomal nondisjunction during meiosis or Robertsonian translocations of parental origin are at the molecular core of the syndrome. The incidence of Down syndrome is estimated at 1:800-1:1.000 live births and it occurs in all ethnic and social groups. Due to the fact that there are physiological and genetic restrictions in patients with Down syndrome that cannot always be accepted by the parents, in many countries they are offered the possibility of pregnancy termination in case of trisomy 21. In the case of twin pregnancy with only one fetus with aneuploid karyotype, a selective termination of that fetus is possible. The time of the procedure does not statistically and significantly increase the risk of premature birth of the healthy twin [2].

Proper prenatal screening, focused on early diagnosis of the problem, followed by specialist care, family involvement, education and training since early childhood, have critical influence on the quality of life and final stage of development of children with Down syndrome, as well as peer acceptance and family functioning.

The standard of conducting prenatal screening for trisomy 21 during the first trimester (between 11–13 weeks + 6 days) combines the evaluation of fetal nuchal translucency and quantitative analysis of the PAPP-A protein, and free β-hCG. That recommendation is based on studies by Nicolaides et al., and is characterized by high sensitivity and specificity, reflected by a 90% detection rate of Down syndrome [3]. Twin pregnancies constitute a separate issue of prenatal screening. In healthy twin pregnancies, the PAPP-A protein concentration and free β-hCG determined in blood serum of pregnant mothers reach higher values when compared to a singleton pregnancy [4], supposedly due to the volume and function of placenta since PAPP-A is synthesized primarily by syncytiotrophoblast [5].

As a result, separate reference values for biochemical parameters need to be acquired in multiple pregnancies. Spencer [4] showed that average PAPP-A protein and free β-hCG concentrations in twin pregnancies are 1.860 and 2.099 times higher, respectively, when compared to a singleton pregnancy. Taking that into account and using statistical modeling techniques, he predicted that, at a 5% false positive rate, the detection rate of trisomy 21 in twins will be at the level of 52%-55% based only on biochemical markers.

In contrast to maternal serum biochemistry, the use of ultrasound markers in multiple pregnancies allows to determine the individual risk for each of the twins. With selective application of the NT measurement and appropriate estimation of the risk, the detection rate is 75%. The combination of these parameters indicates an 80% detection rate of trisomy 21 in twin pregnancies, which is only about 10% lower than for a singleton pregnancy.

Case report

We present a case of a 33-year-old pregnant patient with regular menstrual cycles lasting 28 days. The pregnancy was estimated to be 5 weeks and 4 days, calculated from the last menstrual period. A transvaginal ultrasound revealed the presence of a intrauterine pregnancy with gestational sacs. At 9 weeks of gestation, a diamniotic, dichorionic pregnancy, with symmetrically developing fetuses, was confirmed. Two weeks later a diamniotic/dichorionic pregnancy was reconfirmed by the first trimester ultrasound, with symmetrical CRL values but also with significant asymmetry of the NT values between the fetuses.

The value of fetal NT, whose cytogenetic analysis of the material indicated aneuploid female karyotype 47, XX, +21, was 6.3 mm (Fig. 1, 2). The NT of the fetus with the normal karyotype was 1.8 mm. The analysis of the consecutive markers of fetal chromosomal defects, performed in accordance with the FMF criteria, including the nasal bone (NB) (Fig. 3) assessment, blood flow at the level of the tricuspid valve (TV), and the ductus venosus (DV) (Fig. 4), allowed to calculate the risk of trisomy 21, 18 and 13.

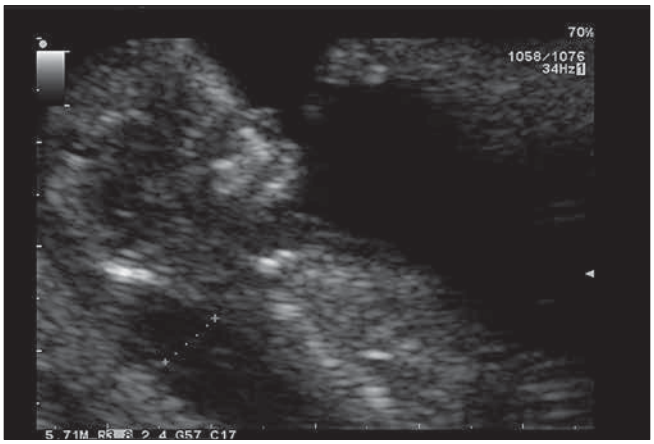
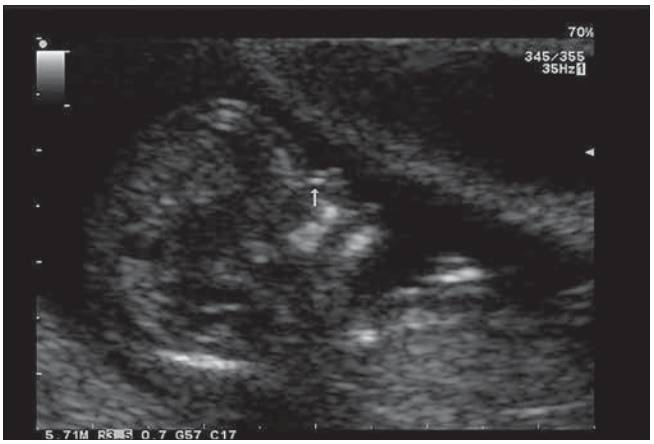


Fig. 1.



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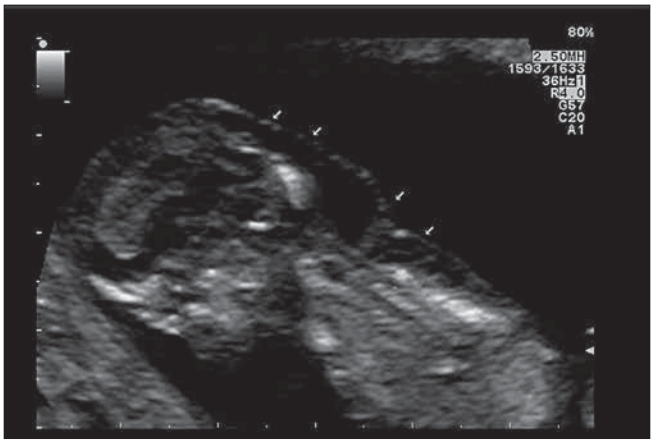
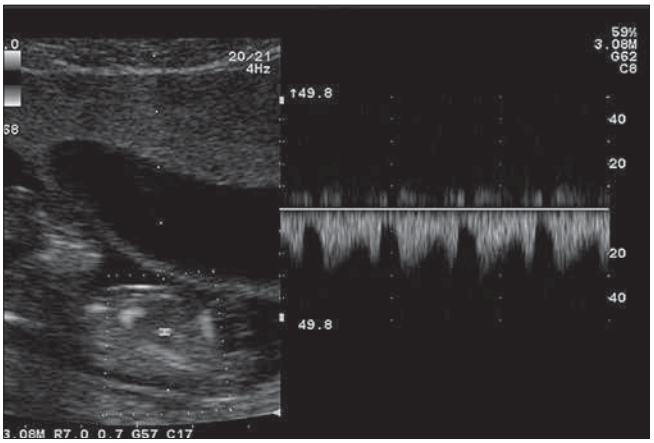


Fig. 2.



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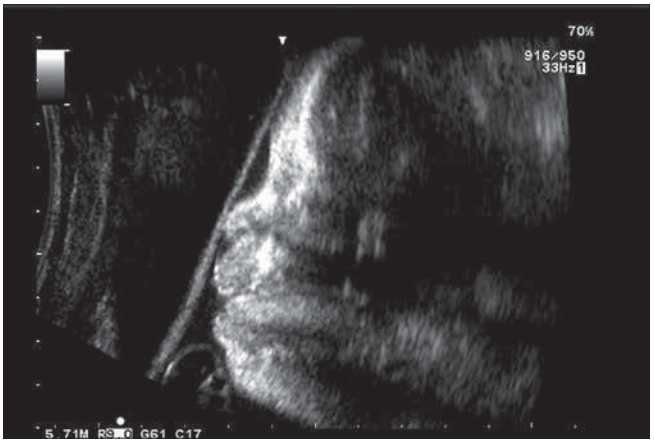

Fig. 5.

Given that the highest achievable level of detection of fetal trisomy 21, 13 and 18 is provided by a screening test performed during the first trimester, combining the analysis of the ultrasound markers (NT, TV, DV) and determination of PAPP-A proteins and free β-hCG serum concentration, the patient was asked to have a blood analysis performed to comply with the test. A written consent was obtained from the patient.

The final risk is calculated based on maternal age. The new risk is the risk calculated on the basis of fetal chromosomal aberration markers - biochemical and ultrasound. Median biochemical values are adjusted to maternal body weight, parity, and twin pregnancy. New risks are calculated with the FMF software version 2009.

As continuation of diagnostic evaluation, the patient was offered amniocentesis after 15 weeks of gestation. The material obtained in the course of this invasive procedure helped to identify that the first fetus (Fetus I) carried a normal male karyotype - 46, XY, whereas the cytogenetic analysis of the material from the second fetus (Fetus II) revealed an abnormal female karyotype - 47, XX +21 (Fig. 5).

The patient decided to continue with the pregnancy and its course was uneventful until the end of 36 weeks of gestation. At 37 weeks of gestation a cesarean section was performed due to spontaneous onset of labor and transverse position of one of the twins. Two newborns were delivered: male (weigh 3060 g, Apg 10) and female (weight 2400, Apg 8). The newborn diagnosed with an aneuploidal female karyotype 47, XX ,+21 manifested reduced muscle tension and dysmorphic features characteristic for Down syndrome, such as flat profile, protruding tongue

(glossoptosis), single crease across the palms and small and low set ears.

The mother and both infants were discharged on day 15 of hospitalization in good overall condition.

Discussion

The risk of having a child with Down syndrome is 1:1490 for mothers aged 20-24; at the age of 40 the risk is 1:60, and >49 years of age it increases to 1:11 live born infants. The risk increases with maternal age, however 80% of children with Down syndrome are delivered by mothers <35 years, which is associated with the highest percentages of deliveries in that age group [3].

Recent data suggests that father's age also plays a role in increasing the risk of Down syndrome in the offspring, through the mutagenic effect of biological and environmental factors in the gametes. Additionally, older males are also assumed to be in relationships with their age peers, what needs to be taken into consideration in the studies [6].

The risk of chromosomal abnormalities in twin pregnancies is higher compared to a singleton pregnancy. Determination of zygosity helps to select the right algorithm for calculating the risk of aneuploidy. Generally, three clinical situations may occur: one fetus with chromosomal anomaly in dizygotic pregnancy, both fetuses with chromosomal anomaly in dizygotic pregnancy, and both fetuses with equal chromosomal anomaly in monozygotic twin pregnancy.

In the past, gender differences in twin pregnancy demonstrated in the course of diagnostic ultrasound, could constitute an argument proving dizygosity. However, in recent years, different studies have indicated phenotypic as well as genotypic differences in identical twins. At their base the intrauterine environmental factors play a major role, especially the molecular mechanisms causing such variable degree of genetic mosaicism in the fetuses [7].

Most cases of twin pregnancies are dizygotic, and each of the fetuses, a priori, has its own risk of developing birth defects. Under the rules of probability, a chance of detecting the defect in at least one of the fetuses is higher than in a singleton pregnancy. According to this thesis, patient at the age of 33 in twin pregnancy will face the risk of Down syndrome in at least one of the fetuses comparable or equal to that of a 35-year old woman in a singleton pregnancy [8]. Thus, a question about implementation of appropriate procedures for women <35 in a multiple pregnancy has been raised.

In the presented case, prenatal care scheme was based on ultrasound diagnosis. The importance of ultrasound screening performed in the first trimester is even more important in case of multiple pregnancies.

First trimester is the optimal time to determine chorionicity and amniocity [9, 10, 11]. Different thickness of the intertwine membrane ('lambda sign' or 'twin peak') at the point of connection with the chorionic plate shows that the pregnancy is dichorionic. If the membrane is thin over its entire length, also at the junctional point with the chorionic plate (a 'T' sign), it is possible to diagnose monochorionic and diamniotic pregnancy with high probability.

The knowledge of chorionicity is important for selecting proper prenatal care as monochorionic/diamniotic pregnancies are associated with significantly higher risk of complications than dichorionic /diamniotic pregnancies. Sonographic determination of chorionicity and the measurement of fetal NT in both fetuses have become a key element of prenatal diagnosis in a twin pregnancy.

It should be strongly emphasized that in monochronic / diamniotic pregnancies the risk of twin to twin transfusion syndrome (TTTS) significantly increases if there are differences in the NT values and in the analysis of blood flow at the level of DV – the reversal of the wave in one of the fetuses increases the risk of TTTS - between the fetuses [12, 13].

Using biochemical markers to assess the risk of aneuploidy in multiple pregnancies is a valuable addition to ultrasound diagnosis. Unfortunately, these parameters are not conclusive enough to assign the risk to a particular fetus. There are not many studies investigating the concentration levels of PAPP-A and B-hCG in multiple pregnancies with fetal aneuploidy and they often comprised groups with small number of patients [14].

Spencer, when testing and verifying the concentrations of PAPP-A and B-hCG in multiple pregnancies, demonstrated the total average of MoM adjusted for weight, ethnicity, smoking, and in vitro fertilization, among twin pregnancies, to be 2.023 for β-hCG and 2.121 for PAPP-A [15]. Considering chorionicity, among mono and dichorionic twin pregnancies, mean MoM values for β-hCG, adjusted for weight, ethnicity, smoking and in vitro fertilization, did not significantly differ from each other and were 1.983 and 2.041, respectively. However, the average value of MoM for PAPP-A, adjusted for weight, ethnicity, smoking and in vitro fertilization, was significantly lower in monochorionic than in dichorionic twins, and amounted to 1.756 and 2.250, respectively [15].

Maternal serum markers in higher order multiple pregnancies (triple or higher) are not useful due to a greater number of possible chorionicity types.

There are many benefits to first trimester ultrasound screening. Firstly, the combined ultrasound and analysis of biochemical markers (PAPP-A, free β-hCG) give the highest, currently achievable level of detection of aneuploid fetuses [3, 16]. Secondly, measurement of NT is helpful in determining the risk of a number of anomalies other than aneuploidy [17, 18, 19]. Thirdly, almost complete assessment of the fetal anatomy can be performed as early as the first trimester [20, 21], thus providing the pregnant woman with a large amount of valuable information at a very early stage of pregnancy. If a fetal defect is indeed recognized, such diagnostic system offers the parents maximum privacy and independence regarding their decisions about the pregnancy. Finally, the first trimester ultrasound screening includes a measurement of CRL, which is the most reliable biometric parameter determining the duration of pregnancy.

Oświadczenie autorów:

1. Józef Krawczyk – autor koncepcji i założeń pracy, przygotowanie manuskryptu i piśmiennictwa– autor zgłaszający i odpowiedzialny za manuskrypt.
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4. Krzysztof Drews – współautor tekstu pracy, korekta i aktualizacja literatury.
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Konflikt interesów:

Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

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