

TRIPLOID KARYOTYPES IN PRENATAL DIAGNOSIS AT UNIVERSITY CLINICAL CENTER OF REPUBLIC OF SRPSKA

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Abstract

Triploidy is chromosomal abnormality characterized by the presence of three sets of chromosomes instead of the normal two sets, so the triploid fetus has 69 chromosomes. Within the Department for medical genetics at University Clinical Center of Republic of Srpska, from 2009 to 2016, 5853 prenatal karyotypizations were performed from amniotic fluid. Two cases (0,03%) of triploid karyotype were detected during this period. In this report we will present the findings of triploid karyotype 69, XXX in prenatal diagnosis. Origin of those triploid karyotypes was determined using molecular methods at Institute of Forensic Medicine of Republic of Srpska, Banja Luka. One of the detected triploid fetuses originated from twin pregnancy, where the pregnancy was spontaneously aborted two weeks after amniocentesis. Using molecular methods, it was determined that one set of chromosomes originated from the mother, and two sets from the father-diandric origin. The other triploid fetus originated from one fetus pregnancy, where the pregnancy was spontaneously aborted two weeks after amniocentesis. The first trimester combined test indicated triploid karyotype with digyny origin. Triploid karyotype was confirmed by prenatal karyotypisation, while molecular analysis confirmed digyny origin of this case of triploidy – two sets of chromosomes originated from mother and one set from father. Since cytogenetic analyses are performed from amniotic cells at 16-18 weeks, in order to reduce waiting period, parents should be informed about karyotypisation and DNA profiling from chorionic villi sample at 11-12 weeks as a faster option to get final results.

Key words: triploidy, prenatal diagnosis, ultrasound, karyotyping, STR markers

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Introduction

Triploidy is chromosomal abnormality characterized by the presence of three haploid sets of chromosomes instead of the normal two sets, so the triploid fetus has 69 chromosomes. Triploidy can occur after fertilization of diploid oocyte with haploid spermatozoon or haploid oocyte with diploid spermatozoon. Diploid sperm and diploid ovum

result from failure of meiotic division. Another, more frequent model is fertilization of haploid oocyte with two haploid sperms. The most frequent karyotype in triploid is 69,XXY, while 69,XXX karyotype is present with slightly lower frequency, and 69,XYY in only 3% (Vladareanu et al., 2006). It has been suggested that 69,XYY triploid conceptus are incompatible with significant embryonic development (Gersen et al., 2005).

Triploidy affects about 1% of recognized conceptions, but it is highly lethal and rarely observed in live births. The prevalence at 12 weeks gestation is about 1 in 2,000 and this ratio drops to 1 in 250,000 by 20 weeks (Jacobs, 1978, Snijders, 1995). About 17-25% of all chromosomally aberrant abortuses are affected by triploidy (Vladareanu et al. 2006, Gersen et al. 2005). But, the last data of another authors shows that incidence of liveborn infants with triploidy is 1 on 100,000 newborns (Chen, 2006). So, close to 100% of the time, triploid aborts spontaneously, but in some cases not until the pregnancy is well advanced (Gardner and Sutherland, 2004). Occurrence of triploid pregnancy does not show correlation with maternal age (Gersen et al., 2005).

There are two distinct phenotypes of triploid, depending on whether the origin of the extra haploid set is paternal (diandric origin) or maternal (digynic origin) (Gersen et al., 2005). Diandric origin of triploid is characterized by well-grown fetus with or without microcephaly and abnormally large and cystic placenta usually classified as partial hydatidiform moles (Gersen et al., 2005). The fetal nuchal translucency (NT) tends to be high and maternal serum-free β -hCG is about 10 times higher than normal. Diandric triploid can cause severe maternal complications, including severe early-onset preeclampsia and choriocarcinoma (Vladareanu et al., 2006).

The digynic type is characterized by a small normal looking placenta, severely growth-restricted fetus with pronounced wasting of the body and sparing of the head, normal fetal NT thickness and very low serum-free β -hCG and PAPP-A (Vladareanu et al., 2006).

The findings suggest that diandric triploids survive longer than digynic triploids (Schinzel A, 2001). More than 50 cases of nonmosaic triploidy have been reported in liveborns. Patients die shortly after birth. This being so, the offer of termination is appropriate when triploidy is diagnosed (Gardner and Sutherland, 2004).

Patients with diploid/triploid mixoploidy (mosaic triploidy) are less severely affected than nonmosaic and can survive beyond 10 years. Clinical features

include intrauterine growth retardation, hypotonia, craniofacial anomalies, syndactyly, malformation of the extremities, adrenal hypoplasia, cardiac defects, and brain anomalies (Schinzel A, 2001).

Prenatal diagnosis

First-trimester combined screening for trisomies 21, 18 and 13 by fetal NT and serum-free β -hCG and PAPP-A has the beneficial side effect of detecting about 85% of fetuses with triploidy (Vladareanu et al., 2006). A cohort study of 198,000 pregnant women in Danish population showed that detection rate of triploidy by first-trimester screening was 83.3% (Engelbrechtsen, 2013). After an indication of chromosomopathy by ultrasound and biochemical screening, karyotype of fetus is determined from chorionic villi or amniotic fluid. Chorionic villi sampling is performed at 11-12 gestational week, and amniocentesis is performed from 16-18 gestational weeks. During genetic counseling at focus are emotional turmoil suffered by the family when first-trimester combined screening shows a triploid fetus, and waiting period for confirmation of the indication by cytogenetic methods. Sarno et al. (1993) reported a unique case of complete placental/fetal discordance with triploidy on chorion villi sample (CVS) and a normal diploid karyotype on amniocentesis and fetal blood sampling, with the birth of a normal baby; such a possibility warrants consideration where triploidy on CVS accompanies an ultrasonographically normal fetus. Nonmosaic triploidy typically shows ultrasonographic anomalies (Gardner and Sutherland, 2004).

Origin of triploidy is determined by analysis of short tandem repeats (STR analysis) using molecular methods.

Materials and methods

Samples of amniotic fluid obtained by amniocentesis were cultured in a complete medium in an incubator at 37 °C, with 5% CO₂ for a period of 10-15 days. When the mitotic index in the culture was assessed to be high enough and that a large number of cells were in metaphase, the division was stopped by the addition of a cytostatic - colcemid. After 2 hours and 30 minutes of adding colcemid, samples were

processed by standard technique for chromosome preparation. Karyotype analysis was performed on 16 metaphases. Eight metaphases were analyzed by GTG bands with resolution of 400 to 550 bands and the other 8 metaphases were analyzed by conventional method. Karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2005, ISCN, 2013).

DNA analysis was performed and DNA profile obtained in RS Forensic Medicine Institute DNA laboratory. DNA analysis was performed on two types of samples:

1. Referent blood sample from both sets of parents,
2. Amniotic cell culture from both fetuses.

From the both types of samples, DNA extraction was carried out using the QIAamp micro-kit for DNA isolation, manufacturer Qiagen, USA. DNA fragments were amplified using the "PowerPlex ESX 16" forensic analysis kits, manufacturer „Promega“, USA, on the "ABI-Veriti" device, manufactured by Applied Biosystems, USA. Detection of DNA amplified fragments was performed by capillary electrophoresis method on the "ABI Prism 310 Genetic Analyzer", manufactured by Applied Biosystems, USA. Analysis of the obtained results was performed using the software package "GeneMapper ID v3.2.1.", Manufactured by "Applied Biosystems", USA. (Figure 2, 5).

Results and Discussion

One of the detected triploid karyotypes in prenatal diagnosis in Department for medical genetics at University Clinical Center of Republic of Srpska was from twin pregnancy. Due to mother's age, amniocentesis was the first prenatal test. Amniocentesis was performed at the 20th gestational week. Only sample from one twin was sent to our laboratory. After two weeks we described karyotype of the fetus as triploid karyotype 69,XXX (Figure 1). Meanwhile, the pregnancy was spontaneously terminated, and we did not get any information about physical status of aborted fetuses.

In order to determine the parental origin of triploidy we performed DNA analysis from amniotic cells,

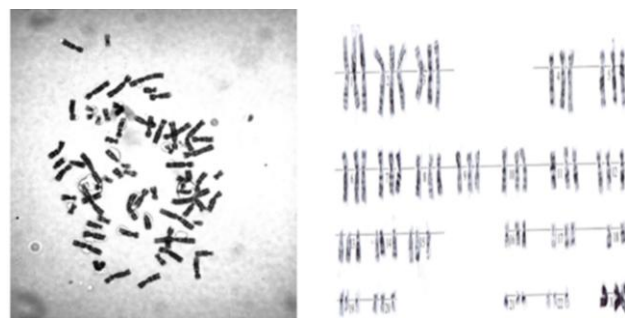


Figure 1. Karyotype and karyogram of the fetus with triploidy - 69,XXX (first case)

and from peripheral blood of mother and father (Figure 2). DNA analysis of amniotic cell culture determined the female DNA profile (Table 1). At 4 out of 15 loci this DNA profile has combination of 3 alleles. In all 4 cases two alleles are identical to father's alleles, and one allele is identical to mother's allele. At 10 loci in this DNA profile there are two alleles that are of different height. The higher one corresponds to the combination of one mother's allele and one father's allele or two identical father's alleles, and the lower allele corresponds to father's allele or mother's allele. At one of the examined 15 loci there is a homozygous variant - one allele that corresponds to the combination of one mother's and two father's alleles. STR DNA analysis showed that fetal karyotype 69,XXX was consequence of conjugation of haploid ovum with two haploid spermatozoa.

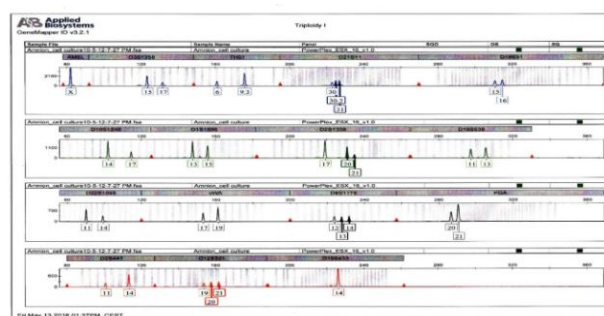


Figure 2. Electrophoregram of the fetus with accentuated triple allele (case 1)

Another triploid karyotype detected in our Department was from one fetus pregnancy. Indication of triploid karyotype of digyny type was set during first trimester combined test at gestational age 11 weeks+5days. Biochemical markers were significantly low (β -HCG and PAPP-A) and relative macrocephaly with antero-posterior diameter about

half of the crown-rump length. Nuchal translucency and nasal bone were without significant deviation. Placenta was posterior low and small (Figure 3).



Figure 3. Fetus with triploidy at 9 weeks of gestation without significant markers of triploidy (above) and fetus with triploid karyotype at gestational age 11 weeks+5 days (beneath)

Amniocentesis was performed at the 17th gestational week. Cytogenetic analysis showed triploid karyotype 69,XXX (Figure 4). DNA profiling from amniotic cells, and peripheral blood of mother and father showed digynic origin of triploidy in this case (Figure 5). DNA analysis of amniotic cell culture determined female DNA profile (Table 2). At 8 out of 15 loci this DNA profile has combination of 3 alleles. In all 8 cases two alleles are identical to mother's alleles, and one allele is identical to the father's allele. At 6 loci two alleles of different height are detected. The higher one corresponds to the combination of one father's allele and one mother's allele or two identical mother's alleles, and the lower allele corresponds to father's allele or mother's allele. One of the examined 15 loci has a homozygous variant - one allele that corresponds to the combination of one father's and two mother's alleles. STR DNA analysis showed that fetal

karyotype 69,XXX was consequence of conjugation of diploid ovum with one haploid spermatozoa.

Table 1. DNA profiles of mother, father and amniotic cell culture

lokus	mother (M)		father (F)		child - amniotic cell culture		
Amel.	X	X	X	Y		X	X
D3S1358	15.0	16.0	15.0	17.0		15.0	17.0
TH01	6.0	7.0	7.0	9.3		6.0	9.3
D21S11	30.0	31.0	30.2	31.0	30.0	30.2	31.0
D18S51		15.0	16.0	17.0		15.0	16.0
D10S1248	14.0	16.0	14.0	17.0		14.0	17.0
D1S1656	15.0	16.3	12.0	13.0		13.0	15.0
D2S1338	17.0	25.0	20.0	21.0	17.0	20.0	21.0
D16S539		11.0	13.0	14.0		11.0	13.0
D22S1045	14.0	16.0		11.0		11.0	14.0
vWA	17.0	18.0	14.0	19.0		17.0	19.0
D8S1179	10.0	12.0	13.0	14.0	12.0	13.0	14.0
FGA	20.0	24.0		21.0		20.0	21.0
D2S441	11.0	12.0	11.0	14.0		11.0	14.0
D12S391		19.0	20.0	21.0	19.0	20.0	21.0
D19S433		14.0	14.0	15.0			14.0

Triploidy is very rarely encountered in prenatal diagnosis because triploid pregnancies spontaneously terminate at early gestational age. If fetus survives until the first trimester gynecologist can establish strong indication of triploidy by expert ultrasound. That indication should be supported by first trimester combined test. Cytogenetic analysis from chorionic villi sample (at 11-12 gestational age) or from amniotic cells obtained by amniocentesis (16-18 gestational week) finally confirm triploid karyotype.

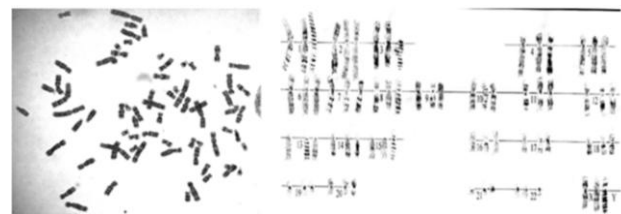


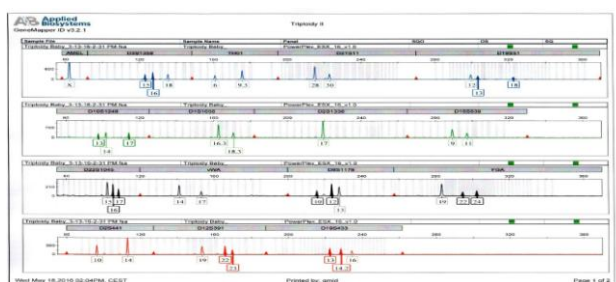
Figure 4. Karyotype and karyogram of the fetus with triploidy - 69, XXX (second case)

In our first mentioned case, triploid karyotype 69,XXX was detected from amniotic cells. Indication for prenatal diagnosis was mother's age. DNA profiling showed diandric type of triploidy - two sets of chromosomes were from father and one

Table 2. DNA profiles of mother, father and amniotic cell culture

lokus	mother (M)		father (F)		child - amniotic cell culture		
Amel.	X	X	X	Y		X	X
D3S1358	15.0	16.0	16.0	18.0	15.0	16.0	18.0
TH01	6.0	9.3	9.0	9.3		6.0	9.3
D21S11	28.0	29.2		30.0		28.0	30.0
D18S51	13.0	18.0	12.0	14.0	12.0	13.0	18.0
D10S1248	13.0	17.0	13.0	14.0	13.0	14.0	17.0
D1S1656		16.3	17.0	18.3		16.3	18.3
D2S1338		17.0	17.0	21.0			17.0
D16S539		9.0	11.0	12.0		9.0	11.0
D22S1045	16.0	17.0	14.0	15.0	15.0	16.0	17.0
vWA	14.0	17.0	14.0	15.0		14.0	17.0
D8S1179	10.0	12.0		13.0	10.0	12.0	13.0
FGA	22.0	24.0	19.0	22.0	19.0	22.0	24.0
D2S441	11.0	14.0		10.0		10.0	14.0
D12S391	22.0	23.0		19.0	19.0	22.0	23.0
D19S433	13.0	14.2	14.0	16.0	13.0	14.2	16.0

from mother. Diploid sperm could be excluded, because, if there had been a failure in the father's first meiotic division, it would have produced 46,XY sperm (the fetus is 69,XXX). If second meiotic division had failed, an XX sperm would have been obtained, but the sperm would have carried identical alleles on both sets of chromosomes, which had not been the case. It can be concluded that two normal, haploid sperm participated in fertilization.

**Figure 5.** Electrophoregram of the fetus with accentuated triple allele (case 2)

In our second case, an indication of triploid pregnancy – digynic type, was set by gynecologist after the first trimester combined test results suggested further prenatal diagnosis. Karyotypisation from amniotic cells confirm triploid karyotype 69,XXX. STR analysis of amniotic cells and parents' peripheral blood samples in this case

confirmed digynic type of triploidy, i.e. fertilization of diploid oocyte with one haploid sperm.

Conclusions

In Department for medical genetics at University Clinical Center of Republic of Srpska triploid karyotype in prenatal diagnosis from 16th to 18th gestational week was detected with very low frequency – 0,03% of all analyzed samples (5853) of amniotic fluid.

Triploid pregnancy could be recognized in the first trimester by using expert ultrasound. That indication should be supported by first trimester combined test. In order to get genetic confirmation of triploidy, usually cytogenetic analysis is performed from amniotic cells at 16-18 weeks. Although, karyotypization and DNA profiling from chorionic villi sample at 11-12 weeks of gestational age, could be diagnostic choice for confirmation of suspicion to triploidy set by first-trimester screening. In this way, waiting time for final confirmation of triploidy and emotional suffering of pregnant woman and family could be reduced.

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