Original Research

Prenatal Diagnosis By Cytogenetic And Microarray Analysis Of Tetrasomy 9p

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Abstract

Background: Tetrasomy 9p syndrome, is a clinically diagnosable rare cytogenetic disorder characterized by tetrasomy 9p associated with a distinctive pattern of multiple congenital anomalies. Trisomy 9p syndrome is the fourth most frequent chromosome aberration seen in infants. Tetrasomy 9p is a generic term describing the presence of supernumerary chromosome incorporating two copies of the 9p arm.

Aim: Present study was aimed to identify Tetrasomy 9p by Karyotype and array-CGH.

Methods: In this case, the karyotype and microarray analysis were performed from amniotic fluid specimen with gestation age of 28 weeks by using standard procedures.

Results: 29-year-old pregnant woman was diagnosed for live single fetus. The fetal biparietal diameter was 71 mm, head perimeter 254 mm and occipital frontal diameter was 90 mm with tetrasomy 9p.

Conclusion:The fetus born with tetrasomy 9p will not survive till reproductive age; hence parents were suggested for prenatal chromosomal analysis which will help in future pregnancies.

Keywords: Prenatal diagnosis, Tetrasomy 9p, isochromosome, Microarray, Mosaicism.

INTRODUCTION

The 9p tetrasomy (T9p) is a rare chromosomal abnormality defined by the presence of extra chromosome in the form of isochromosomes or isocentric chromosomes derived from the short arm of chromosome 9. It was first described by Ghymers in 1973.[1] A baby with tetrasomy 9p was reported for Dandy Walker Malformation (DWM).[2] DWM is a disorder of cerebellum development and associated with a variety of genetic syndromes and chromosomal abnormalities. Other chromosomal disorders (*i.e.*, trisomy 9, 15, 18, triploid and turner syndrome) were reported having abnormal cerebellum development. DWM has also been associated with maternal diabetes exposure to teratogens. Duplication of 9p is commonly inherited due to a parental reciprocal translocation between chromosome 9 and another autosome; very few cases occur due to de novo non familial aberrations.[3] Two varieties of tetrasomy 9p exist; one is iso-dicentric chromosome 9p (i(9p)), where the two 9p arms are linked by a single centromeric region and another is pseudo dicentric 9p (idic(9p)), where one active and one inactive centromere are linked together by a proximal segment of 9 q that may incorporate euchromatic material. Tetrasomy 9p leads to a variable phenotype ranging from multiple congenital anomalies with severe intellectual disability and growth delay to subnormal cognitive and physical developments. The syndrome has been described within a spectrum of clinical characteristics consisting of craniofacial and digital dysmorphic features, variable levels of mental retardation, and growth delay.[4-5] Hypertelorism, abnormal ears, micro-retrognathia and bulbous nose are the most common dysmorphic traits, microcephaly, growth retardation, joint dislocation, scoliosis, cardiac and renal anomalies were reported in several cases. Here in this report, we presented a case of tetrasomy of 9p which was characterized by karyotyping and array-CGH.

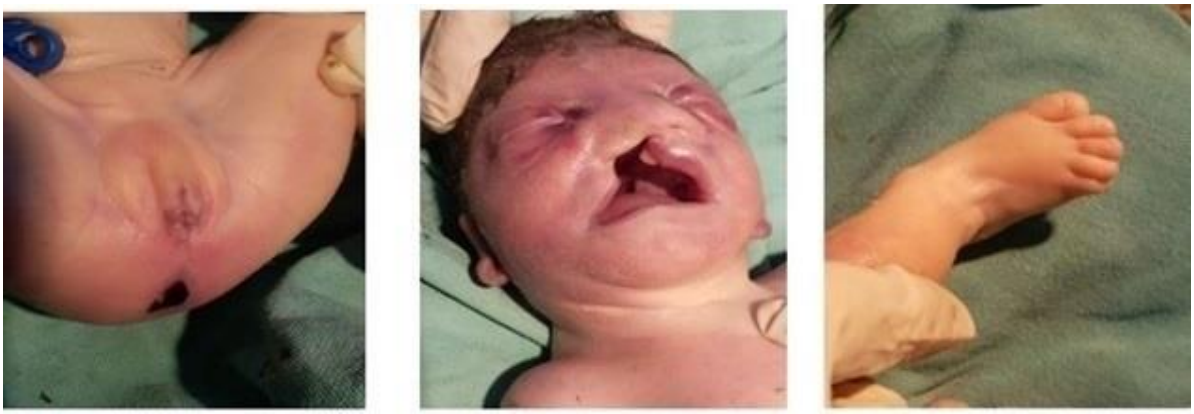

Figure 1: Fetus born with Tetrasomy 9 p

MATERIALS AND METHODS

The present study is the case of a 29-year-old pregnant woman who was referred to our institution, having ultrasonography (USG) and amniocentesis done at 28 weeks gestation period with polyhydramnios abnormalities. The ultrasonography observations were recorded before sample collection. The study of patient who underwent prenatal testing was as per the norms of Institutional Ethics Committee and Pre–Conception and Pre-Natal diagnostic Techniques Act, 1994. The pregnant women received genetic counselling and informed consent was signed by them.

In this case, the karyotype and microarray analysis were performed from amniotic fluid at gestation age of 28 weeks. Chromosomal examination was performed from collected amniotic fluid using a long-term culture method using 2.5 ml of Amniomax (Gibco) culture medium in 25 cm culture flask and incubated at 37°C, 5% CO_2 incubator for 5-7 days. Media change was given 2-3 times during the growth of the culture. After adequate cell growth, Colcemid (10 µg/mL; Gibco,) was

added to the medium flask followed by one hour incubation and transfer to tubes using Trypsin EDTA solution. These harvested cells were treated with 7 ml hypotonic solution (0.56% KCl) and incubated at 37°C for 25 minutes. After incubation, added 3 ml fixative solution and slides were dropped with the cell suspensions. Air drying of the slides was followed by aging at 90°C for 1 hour. After ageing, slides were proceeded for GTG banding. Slides were analyzed by using Cytovision (Leica, Germany).

For microarray analysis, DNA was extracted from amniotic fluid using DNeasy Blood and Tissue Kit (Qiagen). Estimation of gDNA was done by Nano drop (Thermo Fisher) and stored at -20°C.Chromosomal microarray was performed by using standard procedure of CytoScan 750K Array kit (Affymetrix, Santa Clara, CA, USA).

RESULTS

Ultrasonography observations

The patient was pregnant with single live fetus. The skull was evaluated for shape, ossification, and bony defects. A study of intracranial anatomy to assess symmetry, flox-cavum septum pellucidum, and thalamus was included. Facial examination includes examination of the forehead slope, orbits, eyelids, lenses, nasal bones, nasal configuration, upper lip, lower lip, maxilla, mandible, chin, and mandible.

The neck was evaluated in longitudinal, coronal, and axial sections. The chest cavity was evaluated for the chest wall, lungs, heart, mediastinum, and diaphragm. A cardiac examination includes the heart's position, volume, rhythm, chamber view, and outflow tract. Lung length and echogenicity were evaluated. The mediastinum was evaluated for masses and displacements. Diaphragm and hiatuses were searched for anatomic assessment of the abdominal cavity; monitor visceral condition, echogenicity, urinary distention if present, and bladder full and empty phases. Assess the extremities for the presence of bone and soft tissue in the proximal, middle, and distal portions of the upper and lower extremities. Assessment of fetal movement, the study included a genetic sonogram with specific searches for minor and major markers. The placenta was posterior to the upper segment and was normal in grade I. The amniotic fluid index was 236 mm. The umbilical cord was normal and the third ventricle was not dilated. The ratio between the biparietal bone diameter and the nose was 20.88. The biceps diameter of the fetus was 71 mm, the head circumference was 254 mm, and the diameter of forehead and back were recorded 90 mm. The superior cerebellum was found intact, while the inferior cerebellum recorded hypoplastic, showing communication between the cisterna magna and the fourth ventricle. The transverse diameter of the cerebellum was 37 mm and normal. Magna's cistern was 6mm deep and looks good. There was a unilateral cleft palate. Loss of ventricular septal (VSD) echo was present with loss of aorto-septal continuity. The fetal nasal bone was 3.4 mm and hypoplastic. The eyepiece diameter recorded 13mm, the binocular distance was 49mm and the inter-focal distance recorded at 22mm. The scan showed the baby's stomach to be half full and long. Fetal kidney shape, echo pattern and size were normal. The size of the right glove recorded 39x22x23 mm, and the left one was 38x23x23 mm. There was no water flow (hydronephrosis). Abdominal volume and capacity were normal, although dilatation was not observed.

Karyotype

A total of 50 metaphase chromosomes from the GTG band were analyzed by Cytovision Edition (Leica, Germany). An additional iso-dicentric marker chromosome was recorded in all metaphases analysed.

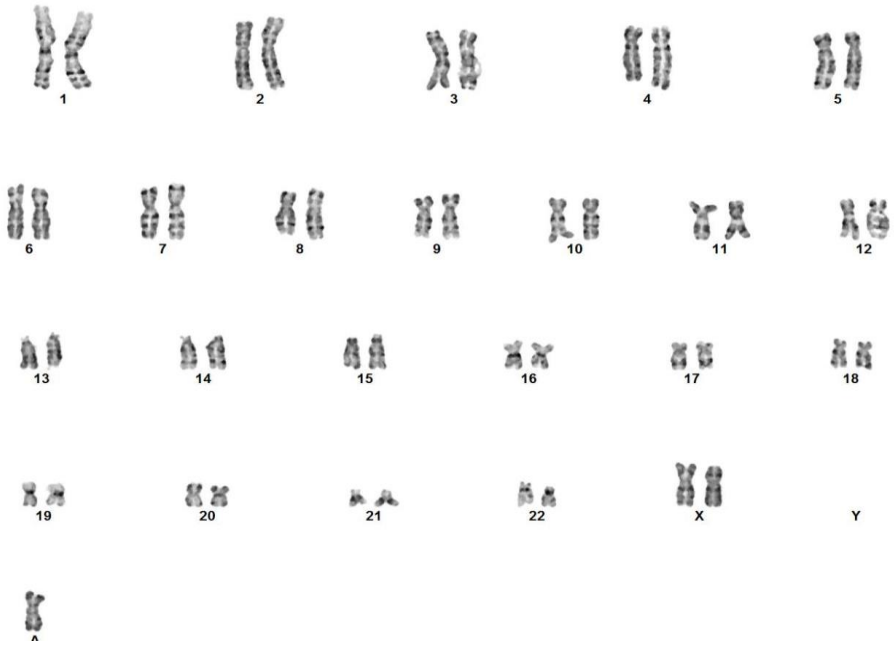

Figure 2. Karyotype

Microarray

Chromosomal microarray analysis shows a tetrasomy of the short arm of chromosome 9 (~47Mb), at position; chr9p24.3p11.1 (1-47,659,533) x3~4 in fetal specimen. The imbalance that results from this chromosomal abnormality is tetrasomy of chromosome 9p. Both karyotyping and chromosomal microarray analysis of the sample show consistent tetraploid results. Analysis of microarray data using Chromosomal Analysis Suite software is shown in Figure 3.

Figure 3. Microarray data analysis by using software Chromosomal Analysis Suite (ChAS) **chr9p24.3p11.1 (1-47,659,533) x3~4**

DISCUSSION

Tetrasomy 9p is a rare structural chromosomal abnormality.[6] Prevalence of supernumerary isochromosomes and iso-dicentric chromosome ranges from 0.4 to 0.72 per 1000 live births.[7] The common these types of abnormalities are i8(p), i9(p), i12(p) or Pallister Killian syndrome, i18(p) and i22(q) or cat-eye syndrome.[8] Except for i(22q), phenotypic expression of these autosomal tetrasomy is often severe.[9] Isochromosomes usually show a tissue restricted mosacismi8(p), found almost only in lymphocytes, i12(p) in fibroblasts,i9(p) in lymphocytes and at lower level in fibroblasts (where it can be absent) as observed whereas the common symptoms of non-mosaic tetrasomy 9p mental retardation and growth retardation and most patients dies during birth or the first year after birth, 54 cases of tetraploid 9p, including fetuses were reported and showing to a variable phenotype ranging from multiple congenital anomalies with severe intellectual disability and growth delay to subnormal cognitive and physical developments.[10-14] Hypertelorism, abnormal ears, micro-retrognathia and bulbous nose are the most common dysmorphic traits.[11-14] Microcephaly, growth retardation, joint dislocation, scoliosis, cardiac and renal anomalies were reported in several cases.[14-15] Those physical anomalies are often, but not universally, accompanied by intellectual disability.[15] Most fetuses with T9p were detected because of the presence of multiple congenital anomalies (MCA) syndrome prompting for fetal karyotyping except one, fortuitously diagnosed following an amniocentesis for advanced maternal age.[16] Based on published reports, the prognosis of T9p is poor: 18/43 patients born alive died during the first year of life, among which 15 died before 3 months. All deceased patients had homogenous T9p in lymphocytes and/or amniotic fluid when T9p was diagnosed prenatally and pregnancy leads to birth.[16-26]

CONCLUSION

The findings of tetrasomy 9p during pregnancy can be extremely variable. The determination of the mosaic situation and the implementation of microarray analysis may provide better genetic counseling.If fetus is born, then it will not survive till reproductive age; hence parents are suggested for prenatal chromosomal analysis which will help in future pregnancies. Early intervention programs for mild developmental delay with minor anomalies and supporting care for severe cases with early detection. We recommend performing appropriate genetic tests in those pregnancies with the suspicion of tetrasomy 9p, evaluating the mosaic state, and following those cases with detailed ultrasonographic examinations.

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LIMITATIONS:

The test results show the variability with standard methods. For numerical chromosomal anomalies there are many techniques available but for the structural chromosomal aberrations, rarely found, can raise different problems for a correct diagnosis and an accurate genetic counseling. Regarding the structural chromosomal anomalies, the most important aspect is about the viability of the fetus, depending on the chromosome(s) involved, the type of anomaly and the size of the defect. For these cases, in some situations, additional investigations should be performed, as a more comprehensive molecular characterization is essential. The genetic investigations must be associated with ultrasound evaluation.

CONFLICT OF INTEREST:

None

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Nil

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