First Croatian Case of Double Aneuploidy: A Child With Klinefelter and Edwards Syndrome (48,XXY,+18) – Possible Causes and Contributing Factors

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ABSTRACT
We report a case of double aneuploidy in a preterm male newborn with karyotype 48,XXY,+18 whose mother was of advanced age and infected with the SARS-CoV-2 virus during the early stages of her pregnancy. The clinical features observed in the newborn included intrauterine growth retardation, dysmorphic facial features, overlapping fingers on both hands, respiratory distress syndrome, ventricular septal defect, patent ductus arteriosus, persistent pulmonary hypertension, and bilateral clubfoot, a phenotype that mainly correlates with Edwards syndrome (trisomy 18). To our knowledge, this is the first reported case of double aneuploidy in Croatia. This paper provides a detailed description of the clinical presentation and treatment strategies used, with the aim of providing valuable data for future recognition and management of similar cases. Furthermore, we discuss the mechanisms of nondisjunction that might account for this rare form of aneuploidy.

KEYWORDS
aneuploidy; COVID 19; genetic nondisjunctions; Edwards Syndrome; Klinefelter Syndrome

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INTRODUCTION

Double aneuploidy refers to the presence of two numerical chromosomal abnormalities in the same individual. The exact mechanism underlying the origin of double aneuploidy remains unclear. Some theories suggest that it may result from either two separate nondisjunctive events during gametogenesis or a single nondisjunctive event in a trisomic zygote (1). The simultaneous occurrence of two chromosomal aneuploidies in a living patient is extremely rare. The first documented case of a patient with double aneuploidy (i.e., 48,XXY,+21 karyotype), was described in 1959 (2). Since then, approximately 400 cases of double aneuploidy have been reported in the scientific literature (1, 3–6). It is probable that the lower frequency of double aneuploidies in the prenatal and postnatal period reflects a higher early intrauterine mortality rate of these fetuses, compared to fetuses with a single chromosome aneuploidy affecting either of the involved chromosome (7). The scarcity of literature data makes it challenging to accurately determine the prevalence of double aneuploidy in all recognized pregnancies.

Autosomal double trisomies are rarely reported in live-born infants and the reported cases of double aneuploidy mostly involve autosomal trisomy and sex chromosome trisomy (1, 8). Most double aneuploidies are associated with increased maternal age, an abnormal sonogram, and early pregnancy loss (1). However, children with sex chromosome aneuploidy and trisomies involving chromosomes 16, 18, and 21 may survive for longer gestation and even the postnatal period (9, 10). While there is limited research on the subject, COVID-19 during pregnancy could result in fetal complications, including intrauterine growth retardation, abortion, preterm delivery, or even stillbirth (11).

The aim of this report is to describe clinical features and management strategies in a patient with double aneuploidy, to review and discuss mechanisms of nondisjunction as a probable underlying cause of aneuploidy, and to discuss the possible influence of COVID-19 parental infection on such chromosomal abnormalities.

CASE REPORT

The mother was 45 years old at the time of the child’s birth. She previously had two uncomplicated pregnancies with normal outcomes and has two healthy children, both born at a gestational age of 41 weeks via spontaneous vaginal delivery.

The mother had a history of regular menstrual cycles, and her last menstrual period occurred two weeks before she contracted COVID-19, at the very beginning of this pregnancy. She experienced relatively mild COVID-19 symptoms, which lasted for two weeks. Symptoms included fever that reached maximum value of 38 °C degrees and lasted up to five days, extreme fatigue, and upper respiratory tract symptoms. She was confirmed positive for SARS-CoV-2 through PCR testing. Her husband presented with similar symptoms at about the same time but was not tested for SARS-CoV-2. The mother also developed hypertension during the pregnancy which was treated with methyldopa. Ultrasound monitoring showed normal fetal anthropometric measurements and proper development up to the 17th pregnancy week. After that, the fetus showed signs of intrauterine growth retardation (IUGR) as well as polyhydramnios. Other fetal characteristics were normal. The mother denied alcohol or drug exposure during pregnancy. The infant was delivered via caesarean section at an estimated gestational age of 33 + 4 weeks due to non-reassuring fetal heart rate (NRFHR), IUGR, polyhydramnios, and pregnancy induced hypertension. The infant’s birth weight was 1230 g (< 3rd percentile), length was 35 cm (< 3rd percentile), and head circumference was 27.5 cm (< 3rd percentile), respectively. APGAR scores were 4 after 1 minute and improved to 6 after 5 minutes. The infant was resuscitated using the T-piece resuscitator. After applying 5 initial sustained inflations lasting for 1 second each, a positive pressure ventilation was continued for 1 minute with a frequency of 40 ventilations per minute, peak inspiratory pressure (PIP) of 20 cmH₂O and FiO₂ rising from initial 21% to 30%. This resulted in an improvement of heart rate to 120 beats per minute and only a slight improvement of muscle tone. However, spontaneous breathing was inefficient and irregular with gasping, so the child was immediately intubated at the beginning of the second minute of life and was placed on mechanical ventilation for appropriate management of respiratory distress. Physical examination revealed several dysmorphic features, including a strawberry-shaped head, a depressed nasal bridge, microretrognathism, low set ears, widely spaced nipples, clenched fists with joint contractions, bilateral clubfoot, and a grade I–II pansystolic murmur. He also had bilaterally undescended testes with a normal size phallus and multiple body hematomas. A neurological exam revealed a hypotonus with normal reflexes. Laboratory findings showed metabolic acidosis, anemia, thrombocytopenia and elevated inflammatory markers, and diagnosis of early neonatal sepsis was made. He was placed, according to an established protocol, on a first-line double antibiotic regimen (β-lactam in combination with an aminoglycoside). A porcine surfactant preparation was administered intratracheally at a dose of 200 mg/kg within 30 minutes after delivery. A 4 F umbilical venous catheter was inserted and its depth was adjusted based on radiography findings. Adequate vascular volume and electrolyte balance were precisely maintained with IV fluids. Additional therapies included: caffeine citrate, calcium gluconate, and sedation with midazolam as well as vasopressor inotropic support. The chest X-ray showed bilateral lung infiltrates, while echocardiography revealed several cardiac abnormalities including the presence of a small muscular ventricular septal defect (VSD), patent foramen ovale (FOA), with a large patent ductus arteriosus (PDA) measuring 5 mm with a predominantly right to left shunt. In addition, the infant had, decreased left ventricular contractility, and supra-systemic pulmonary hypertension of 50 mmHg. An ultrasound of the brain showed no apparent abnormalities. Initially, the child was ventilated using synchronized intermittent positive pressure ventilation with volume guarantee (SIPPV + VG) with optimal adjustment of ventilatory parameters. On day 2, inhaled nitric oxide (INO) at 20 ppm was introduced for...
the treatment of persistent pulmonary hypertension. Due to inadequate therapeutic response and increased oxygen requirements, the conventional ventilation was changed to high-frequency oscillatory ventilation (HFOV) with added inhaled nitric oxide (iNO). Despite receiving maximum respiratory support using conventional mechanical ventilation, the infant’s condition continued to deteriorate, resulting in lethal outcome on fourth day of life. Chromosomal analysis of a peripheral blood sample, which came subsequently, using Giemsa trypsin (GTG banding) showed the karyotype of 48,XXY,+18 (Figure 1). The pathological (autopsy) finding reported a hypotrophic premature male child with the above mentioned dysmorphic and clinical features, bilateral pneumonia and pulmonary hyaloid membrane disease and normally placed internal organs, respectively.

**DISCUSSION**

The coexistence of Edwards and Klinefelter syndromes remains rarely reported in the literature. To the best of our knowledge, this double aneuploidy is the first reported case in Croatia.

Besides the common clinical characteristics in more prevalent Trisomy 18, it is very challenging to diagnose or suspect Klinefelter syndrome when these two conditions coexist.

Our child presented with typical facial features including a strawberry-shaped head, a depressed nasal bridge, microretrognathism, low set ears, widely spaced nipples, clenched fists with joint contractures, and bilateral clubfoot indicating Trisomy 18 but also had cryptorchidism which is commonly seen in both Trisomy 18 and Klinefelter syndrome (11, 12). In Trisomy 18 cardiac abnormalities are commonly seen but not so much in Klinefelter (11); however, in the combination of both trisomies, all the reported cases had cardiac involvement (PDA and VSD in more than 95% of cases) (12–14). Our patient had a grade I–II pansystolic murmur and was found to have VSD, FOA with a large PDA of 5 mm and pulmonary hypertension.

While the prenatal diagnosis remains extremely difficult, findings of polyhydramnios, IUGR, increased fetal nuchal translucency, or structural cardiac abnormality on prenatal ultrasound should cast doubt on the possibility of a chromosomal aberration (13). Consequently, while the coexistence of double aneuploidy is rare, the potential for combined abnormalities involving both autosomal and sex chromosome should be raised even if clinical signs of one of the conditions are not present. Conventional karyotyping was performed on cultured peripheral blood lymphocytes to detect a possible chromosomal anomaly. Thirty metaphases were analyzed to exclude mosaicism and the double trisomy was seen in all analyzed metaphases before concluding the cytogenetic result. Cytogenetic analysis played the main role in establishing the diagnosis of our patient.

Meiosis is the basis of the reproduction process that ensures a reduction of ploidy and promotes genetic diversity in both males and females (15). Female germ cells
undergo the first meiotic division during embryonic development and arrest at the diplotene phase of prophase I before birth. After puberty with each menstrual cycle, some of the arrested oocytes within the preovulatory follicles may resume meiosis in response to luteinizing hormone (LH) surges. Soon after completing the first meiotic division, the second meiotic division starts and the oocyte arrests at metaphase II until fertilization. It is with fertilization only that the oocyte accomplishes its meiosis. Different factors including epigenetic molecules and different signaling pathways have been proven essential for proper meiotic maturation (16).

Regarding the mechanisms of nondisjunction, the parental origin of extra chromosomes and cell division level where nondisjunction occurred were not proved in our case. However, there is a clear association in literature between double aneuploidies and advanced maternal age, as was in our case. The extra chromosomes are mostly of maternal origin and the nondisjunction events can occur in different cell division stages (4, 17, 18). Furthermore, there is a clear association between advanced maternal age and chromosome laxity leading to nondisjunction. This is mainly due to inappropriate chromosome coiling and condensation in the oocyte of older women (19). A two-hit model of nondisjunction has also been suggested, in which the first hit is the prenatal formation of a susceptible tetrad, and the second hit is disruption of the meiotic process that increases the risk of nondisjunction of the susceptible configuration (20). Advanced maternal age remains the only well-documented risk factor for maternal meiotic nondisjunction, but there is, however, a surprising lack of understanding of the basic mechanisms behind maternal age. Recent association studies seem to support the contribution of different signaling pathways.

As the COVID-19 infection broke out in 2019 it has rapidly turned into a global pandemic, becoming a healthcare burden, both for the health system and for patients. More and more questions are being asked about the female reproductive system, fertility problems and pregnancy outcomes, and an explanation is needed about the possible link between COVID-19 and women's reproductive health. In women undergoing IVF post-COVID-19 low levels of vascular endothelial growth factor (VEGF) were found, which could negatively affect the development of ovarian vasculature, reduce the supply of nutrients for the follicles, and lead to poor oocyte quality (26, 27). Moreover, a reduced level of cytokine IL-1β, which regulates folliculogenesis and atresia (28, 29), could also negatively impact oocyte quality (26). Another study compared the IVF outcomes of nine couples before and after COVID-19 infection (30). While the results of the number of oocytes retained and fertilization rates were similar, the number of top-quality embryos (TQE) was significantly lower. TQE was considered an embryo with more than seven blastomeres on day 3, ≤10% fragmentation, and blastomeres of equal size (30). Regarding fertility, the highest influence of SARS-CoV-2 infection was seen in the reduced number and quality of embryos. Chamani et al. (31) evaluated the IVF outcomes of 1881 women who underwent procedures between January and July 2020 to the control group, who underwent procedures in 2019, before the pandemic. The mean number of euploid embryos per patient was significantly lower in May and June 2020 (31, 32). This raises the possibility that, in addition to the advanced maternal age, a recognized risk factor for aneuploidies, parental COVID-19 infection may have played a role in our case of double aneuploidy.

Apart from maternal age, other genetic and environmental factors associated with non-disjunction and aneuploidy remain elusive. Further studies concerning underlying molecular mechanisms could determine causes and contributing factors of non-disjunction errors and bring broader understanding of aneuploidies. Assessing exceptional and ultra-rare cases of segregation failure like ours may be useful in improving our understanding of the general mechanisms of nondisjunction.

ETHICAL APPROVAL

Ethical approval for this case (Ethical Committee No. 2181-147/01/06/M.S.-22-02) was provided by the Ethical Committee of the University Hospital Centre Split (Chairperson Prof. M. Saraga) on May 5, 2022.

REFERENCES


