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40 Years of Experience in Invasive Prenatal Genetic Diagnosis

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Abstract

The field of genetic prenatal diagnosis has seen remarkable advances in terms of prevention of birth defects and genetic diseases in the last 40 years.

This progress is due to the operators' experience with new genome studies and molecular biology analysis.

In the meantime, the most sophisticated ultrasonography equipment has made it possible to perform fetal procedures in early pregnancy as well as in the preimplantation period, thus making prenatal diagnosis increasingly accepted by couples wishing to plan their offspring and reducing the anxiety of waiting for the diagnostic response, and, in severe cases when chosen by the woman, the possibility of terminating the pregnancy in the first trimester.

In the new era of genetic screening using combined tests and noninvasive prenatal testing (NIPT), we have had a decrease in invasive prenatal diagnostic procedures and a progressive increase in chorionic villus sampling (CVS) compared to amniocentesis for chromosomal indications.

In this paper, we describe the invasive sampling techniques performed at Microcitemico Hospital–Cagliari in the last 40 years by fetal blood sampling (FBS), amniocentesis, CVS, and preimplantation genetic diagnosis (PGD) applied to prevent the most common fetal genetic disorders.

Keywords: Amniocentesis, Chorionic villus sampling, Fetal blood sampling, Genetic diseases, Preimplantation genetic diagnosis.

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Introduction

The prevalence of serious birth defects is estimated to be about 5% and includes chromosomal, metabolic, and single-gene diseases.[1]

Many fetal abnormalities and genetic diseases can be predicted and sometimes diagnosed with new screening tests available and the ultrasound examination, but the definitive diagnosis can be obtained by invasive prenatal procedures.[2]

Prenatal diagnosis became routine at the end of the 1970s, and since 1977, we started to perform it in Cagliari, Sardinia using fetal blood sampling (FBS) obtained by placentacentesis. This was the first prenatal diagnosis of β-thalassemia performed in Europe.[3]

In Sardinia, β-thalassemia was a very common genetic disease with 10–12% of carriers. In the 1970s and 1980s, in the absence of prenatal diagnosis, there were 100–200 newborns affected by thalassemia each year.[4]

At the beginning of 1980, we also offered karyotype analysis by amniocentesis and chorionic villus sampling (CVS), and in 2004, preimplantation diagnosis became also available in our center.[5]

The development of ultrasound equipment and new devices allowed us to obtain fetal specimen and placenta tissues with better accuracy and efficacy, implying a very low fetal loss rate.[6]

The progress in molecular analysis has also contributed greatly to offering more accurate diagnoses.[2]

In this paper, we describe the experience of our center in prenatal invasive diagnosis in 60,000 samples by FBS,

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amniocentesis, CVS, and preimplantation genetic diagnosis (PGD) performed for the most common genetic diseases.

Invasive Prenatal Genetic Procedures

All invasive prenatal techniques were performed after nondirective obstetric or genetic counseling and with written consent of the patient.[7]

Information was obtained on genetic risks of anomalies, the options of screening tests, the methods of invasive techniques, the accuracy and efficacy of laboratory analysis of our and other centers, the sampling success, the fetal loss risk, the possible fetal and neonatal therapy, and the maternal choice of pregnancy termination in case of an affected fetus.[1]

All procedures were performed by free-hand techniques under continuous ultrasound monitoring.[8]

Initially, the analysis for β-thalassemia was done by globin chain synthesis analysis by column chromatography[9] and later on by deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) analysis by oligomer technique.[10]

For chromosome diagnosis, we utilized cultured cells from amniotic fluid or from placental tissue and, in several cases, quantitative fluorescent-polymerase chain reaction (QF-PCR) or fluorescent *in situ* hybridization (FISH) for rapid testing and for some common aneuploidies.[2]

The most recently introduced methods such as chromosomal microarray analysis (CMA) and next-generation sequencing (NGS) were also used in several cases for better accuracy of the test.[11]

Methods and Results

Fetal Blood Sampling (FBS)

Fetal blood sampling (FBS) by placentacentesis for β-thalassemia was performed in 1977 at 18–22 weeks of gestation, and for 4–5 years, it was the only sampling procedure for β-thalassemia that our center adopted.

Placentacentesis consisted of fetal and maternal blood sampling from the placenta and, in order to distinguish fetal from maternal blood, separation was performed by culture counter.[9]

At the beginning of 1981, we introduced fetoscopy in order to obtain pure fetal blood samples.[8]

Fetoscopy was performed under the guidance of optical fiber instrument connected to the needle and introduced in the umbilical fetal cord *via* maternal abdomen and with local analgesia.[8]

In 1982, we introduced cordocentesis, which has been the only FBS method utilized since.

Cordocentesis was done under continuous ultrasound monitoring, and it implies introducing through the maternal abdomen a spinal needle (20- or 22-gauge) into the umbilical cord between 18 and 22 weeks.[8]

The needle was introduced perpendicularly to the scanner by free-hand technique.

About 3,000 FBS were performed for β-thalassemia or for other single gene defects and/or to obtain rapid karyotype results in malformed fetuses.

The success sampling was high (98%), and the fetal loss rate was 6, 3.5, and 2% by placentacentesis, fetoscopy, and cordocentesis, respectively.

Amniocentesis

We performed amniocentesis for karyotype study at the beginning of 1980 only for maternal age indication for chromosomal analysis and also using oligomer technique by PCR-DNA analysis for 200 cases of β-thalassemia.[10]

In 1995, following combined screening test (fetal nuchal translucency and biochemistry) and, more recently, with the introduction of noninvasive prenatal testing (NIPT) using cell-free DNA, our center witnessed a trend of decrease in amniocentesis and an increase in CVS.[12,13]

Amniocentesis was also utilized in malformed fetuses and congenital infection cases.

Amniocentesis was performed free-hand under continuous ultrasound monitoring and introducing a spinal needle of 20- or 22-gauge perpendicularly to the scanner. The success at the first insertion was 99 and 100% in two or three insertions, respectively.[8]

We have performed about 30,000 amniocentesis procedures between 15 and 20 weeks of gestation with fetal loss risk <0.5%.

Chorionic Villus Sampling (CVS)

Chorionic villus sampling (CVS) was first performed in our center in 1983. Initially, we used the transcervical (TC) route, but since 1985, the only route we have adopted has been the transabdominal (TA) one, which was the technique generally better accepted by women.[14]

We performed TC-CVS by free-hand technique at 10–13 weeks using a rigid biopsy forceps under ultrasound monitoring in >700 procedures, mostly for β-thalassemia diagnosis.[15]

The forceps were introduced in the chorion frondosum not more than twice after a careful disinfection of the vaginal area. As the high risk of β-thalassemia is 25%, the success of sampling was higher about 100% and fetal loss risk 3%.

In 1986, in order to decrease the risk of fetal loss and infections and to be able to offer early diagnosis before 10 weeks as well as after 14 weeks to those patients who reached our center late[16,17] due to our high experience in TA FBS and amniocentesis, we passed to transabdominal chorionic villus sampling (TA-CVS).[18]

Transabdominal chorionic villus sampling (TA-CVS) was performed by free-hand technique, introducing the 20-gauge spinal needle perpendicularly to the placenta under continuous ultrasound monitoring at any time of gestation.[19]

In few cases, we performed TA-CVS very early (6–9 weeks) and also following 14 weeks for patients at high genetic risk.

In the last 10 years, we opted for 18-gauge spinal needle because it has proved to be very useful for tutoring trainees and implies low fetal loss and a bigger amount of villi collected. It also reduces the necessity of a second or a third needle insertion.[20]

In cases of completely retroverted uterus with posterior placenta (2–5%), transvaginal manipulation of the uterus by the obstetric assistant was required to enable the introduction of the needle in the correct direction.[21]

The needle was connected by a 2-mL syringe, heparinized to avoid clots, with continuous manual syringe aspiration with up-and-down movement for 5–10 seconds.[19]

No anesthesia, analgesia, or antibiotics were applied, the maternal abdomen was first cleansed with an antiseptic solution, and the scanner probe was wrapped up with sterile drape or glove. We have had no sampling failures, and the fetal loss risk was very low, <0.5% in about 30,000 cases.

Preimplantation Genetic Diagnosis (PGD)

Preimplantation genetic diagnosis (PGD) is a very early form of prenatal diagnosis. It was introduced in our center in 2004 for the diagnosis of single gene disorders in those couples who did not want to terminate the pregnancy with affected fetuses after traditional invasive prenatal diagnosis.[22]

The PGD was performed using assisted reproductive technique by *in vitro* fertilization, intracytoplasmic sperm injection, blastomere or blastocyst biopsy, and molecular analysis.

In the beginning, PGD was offered to infertile couples at high genetic risk, and subsequently, it was extended to all couples at such risk. Its acceptance rate was very high, mostly in those couples who have already had a previous termination of pregnancy.[23]

Conclusion

In the last 40 years, our center has been undergoing remarkable changes in invasive prenatal procedures, cytogenetic analysis, and molecular analysis. The most considerable developments have been the earlier samples in the first trimester by TA-CVS and in preimplantation period by PGD. These two techniques have allowed fetal diagnosis to become increasingly more acceptable for couples at high genetic risk. The acceptance rate of FBS, amniocentesis, and CVS was 93.2, 96.4, and 99.3%, respectively.[24]

In the meantime, the continuous progress in molecular biology has brought about higher accuracy and efficacy in the field of prenatal diagnosis. The informative and nondirective genetic and obstetric counseling have always been fundamental for the couples' decision-making process.

Particularly for β-thalassemia, the voluntary termination of pregnancy following traditional prenatal diagnosis in affected pregnancies was a very common choice (>98%), and the incidence of the β-thalassemia has significantly decreased from 100–120 affected newborns in the 1970s to only 5–7 nowadays.[5]

The introduction of screening programs for aneuploidies in 1995 using nuchal translucency and biochemistry tests and, more recently, NIPT, together with the birth rate decline, caused an important reduction of invasive prenatal procedures.[13]

Array-based copy-number analysis [chromosomal microarray (CMA)] and NGS such as target gene-panel sequencing and whole exome sequencing (WES) are the future challenges and perspectives not only for the clinical practice but also due to the social, economical, and ethical issues they imply.[2]

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