

Analysis of fetal and maternal results from fetal genetic invasive procedures: an exploratory study at a University Hospital

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SUMMARY

Objective: To characterize the indications of pregnant women who sought the Fetal Medicine Services of the Hospital das Clínicas, at the Medical School of the Universidade de São Paulo for performing invasive diagnostic procedures, and to evaluate the results of fetal karyotypes and their pregnancies. **Methods:** A retrospective and observational study on pregnant women who underwent chorionic villus sampling (CVS), amniocentesis, and cordocentesis in the period from February, 2005 to December, 2009. Other diagnostic or therapeutic procedures were not included. The result of pregnancy was obtained by consulting patient electronic records, medical records, and/or telephone call. **Results:** 713 procedures were performed (113 CVS, 340 amniocenteses, and 260 cordocenteses). The main indication for performing invasive procedures was the presence of structural changes in fetuses, followed by increased values of nuchal translucency, and advanced maternal age. Fetal karyotype was altered in 186 cases (26.1%). The 18 trisomy was the commonest aneuploidy followed by the 21 trisomy, X monosomy, and 13 trisomy. There were 4.9% cases of miscarriage, 25.7% cases of stillborn infants, and 13% cases of neonatal deaths. Eight pregnant women opted for legally induced abortion. 99% of pregnant women whose fetuses did not present abnormalities and presented normal fetal karyotype had infants who were born alive.

Uniterms: Karyotype; chorionic villus sampling; fetal blood; fetus.

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INTRODUCTION

Pre-natal screening for aneuploidy started in the 1970s, with maternal age as its main indication¹.

The risk of chromosomal abnormalities increases with the maternal age. In a study that evaluated approximately 89,000 classical amniocenteses, pregnant women aged 35 years or over presented more chromosomal changes than women aged less than 35 years. The frequencies of trisomy 21, trisomy 18, and trisomy 13 for women aged over 35 years were 1/100, 1/454, and 1/1438, respectively. Conversely, the frequencies for women aged under 35 years were 1/591, 1/2862, and 1/4651 for the same aneuploidies². The use of maternal age of 35 years or more as a cut-off point for indicating fetal karyotype research presents a sensitivity of approximately 30%.

In the 1990s, biochemical screening in the second trimester started by evaluating alpha-fetoprotein, beta chorionic gonadotropin (β -HCG), and unconjugated estriol in maternal plasma, with a sensitivity of 60% and a false-positive rate of 5% for trisomy 21³. With the improvement of ultrasound devices and equipment, screening was advanced to the first trimester by nuchal translucency evaluation. The detection rate for Down syndrome through nuchal translucency is 77%, with a false-positive rate of 5%⁴. The association of nuchal translucency with protein dosage in maternal blood, maternal serum free-beta-chorionic gonadotropin (free β -HCG) and pregnancy-associated plasma protein-A (PAPP-A) raises the sensitivity to 90%, with the same false-positive rate^{5,6}. Another benefit of combined screening is the reduction of cases with indication for invasive procedure, reducing the exposure of pregnant women to the risks of such a procedure⁷.

Morphological ultrasound scan can be also used for screening chromosomal anomalies in the second trimester of gestation. Some abnormalities can also be related to aneuploidy, such as ventriculomegaly, facial cleft, cardiopathy, diaphragmatic hernia, nephropathy, omphalocele, shortened limbs, and club-foot^{8,9}.

The definite diagnosis of chromosomal abnormality in the antenatal period is only possible by performing invasive procedures and analysis of the fetal tissue or its components, such as trophoblast, amniotic fluid, and fetal blood¹⁰. Chorionic villus sampling (CVS) presents a risk of total fetal loss varying from 2.3% to 3.7%¹¹⁻¹³. Those rates take into account the early procedure, and as a consequence, the presence in this group of chromosomally altered fetuses that would evolve to spontaneous abortion. The advantage of this examination is the early gestational age of diagnosis. The main disadvantage is confined placental mosaicism, which occurs in approximately 1% of the cases and causes the need to repeat the procedure in another environment (amniotic fluid or fetal blood)^{14,15}. Classical amniocentesis offers a risk of fetal loss of 0.3% to 1.0%¹⁶⁻¹⁸.

Cordocentesis is a procedure that allows for the evaluation of fetal karyotype, besides anemia diagnosis, infections, and fetal hemoglobinopathies. This procedure presents a 1.4% risk of fetal loss¹⁹.

At the Obstetrics Department of the Hospital das Clínicas of the Medical School at the Universidade de São Paulo (HCFMUSP), the Fetal Medicine Services is a reference for cases of fetuses with abnormalities or increased risk for aneuploidies. The service performs invasive procedures for fetal karyotype studies in cases diagnosed at the service and in cases referred to from external services. However, data regarding fetal procedures, karyotype results, and evolution of pregnant women who underwent an invasive procedure have not yet been published, and national data are scarce. Publishing those data is relevant, as it represents a reference service in the city of São Paulo and provides useful information on advice to future pregnant women, at local and national level.

The main objective of this study was to characterize the indications of pregnant women who sought the Fetal Medicine Services of a tertiary center for performing invasive diagnostic procedures for fetal karyotype studies. Secondary objectives were to evaluate fetal karyotype results and their gestations.

METHODS

A retrospective, observational, and cross-sectional study was performed at the Fetal Medicine Services of the Obstetric Department at the HCFMUSP; the study was approved by the ethics in research committee of the institution (CaPPesq – HCFMUSP), No. 0600/09.

Pregnant women who underwent invasive procedures (CVS, amniocentesis, and cordocentesis) were selected in the period from February, 2005 to December, 2009.

Pregnant women were referred from other fetal medicine services or from this institution. After genetic counseling and explanation of the risks related to the procedure, the pregnant woman or her legal guardian signed an informed consent. Before performing the invasive procedure, all pregnant women underwent morphological ultrasonography for evaluation of fetal structure.

Only pregnant women who underwent invasive diagnostic procedures for fetal karyotype (CVS, amniocentesis and cordocentesis) were included. CVS was performed between 11 and 14 weeks, six days of gestation; amniocentesis, from 14 weeks; and cordocentesis, from 19 weeks.

Procedures with other purposes, such as amniotic fluid drainage, placement of drains, and laser or punctures for fetal karyotype collected from other dry secretions were not included.

The survey of pregnant women who underwent invasive procedures was performed by consulting the databank from the Information System of Official Reports

on Obstetrics and Gynecology (Sistema Informatizado de Laudos em Obstetrícia e Ginecologia — SILOG), which is used by the Fetal Medicine Services for ultrasonography scans report, invasive procedures, and fetal and gestation scan results. Postnatal results were obtained by consulting paper-based medical records in the HCFMUSP and in SILOG, and/or by telephone call to patients.

Population features evaluated were: patients' age; indications for performing invasive procedures; number and type of procedures performed (CVS, amniocentesis, and cordocentesis); results of fetal karyotype; and evolution of gestations (legally and non-legally induced abortion; miscarriage defined as gestation loss up to twenty weeks of pregnancy; stillborn, defined as birth of fetus dead after twenty weeks of pregnancy; and neonatal deaths, defined as death until 28 days post-birth)²⁰.

Numerical variables were described as maximum, minimum, average and standard deviation, or median. For the categorical variables, simple and relative frequencies were used.

RESULTS

In the period studied, 713 diagnostic invasive procedures were performed as follows: 113 CVS, 340 amniocentesis, and 260 cordocentesis. Table 1 describes indications for diagnostic invasive procedures performed during the period studied.

Regarding CVS, maternal age for performing the procedure varied from 15 to 45 years, with an average of 32.4 (\pm 7.7) years. The average gestational age was 13.4 (\pm 1.3) weeks of pregnancy.

In the group of pregnant women who underwent amniocentesis, maternal age for performing the procedure varied from 14 to 47 years, with an average of 30.7 (\pm 8.2) years.

The gestational age for performing the procedures varied from 14.3 to 34 weeks, with an average of 20.1 (\pm 3.6) weeks. Among the cases of amniocentesis, a case of maternal death was observed 22 days after the procedure, in a case of high risk to the mother's life with a diagnosis of sickle-cell anemia.

Regarding cordocentesis, maternal age varied from 13 to 48 years, with an average of 28 (\pm 7.5). The average gestational age was of 27.1 (\pm 3.6) weeks of pregnancy.

From those pregnant women who underwent cordocentesis, 53.5% (139/260) had children born alive, 24.6% (64/260) had stillborn children, 20.8% (54/260) had neonatal death, and 1.6% (3/260) opted for legally induced abortion.

Table 2 shows the results of fetal karyotypes from the cases submitted to invasive procedures at the Fetal Medicine Services of the Obstetrics Department at the HCFMUSP in the period studied. The result of pregnancies is shown in Table 3.

Table 4 describes the main fetal abnormalities in fetuses evolving to intrauterine death.

DISCUSSION

The majority of patients who were referred to this Fetal Medicine Services presented morphological fetal changes, considering this was the main reason for performing fetal karyotype invasive procedures, representing 69.8% of indications.

The indication rates for performing the procedures differ from the medical literature, taking into account that structural abnormalities represent up to 12% of cases in most studies^{8,14,21,22}. Such a difference among indications may be due

Table 1 – Indications for performing invasive procedures for fetal karyotype research. HCFMUSP, 2005 to 2009

Indication	CVS (n = 113) n (%)	Amniocentesis (n = 340) n (%)	Cordocentesis (n = 260) n (%)
Fetal malformation	26 (23.0%)	227 (66.8%)	245 (94.3%)
Increased NT	64 (56.7%)	29 (8.5%)	03 (1.2%)
Maternal age	20 (17.7%)	49 (14.4%)	04 (1.5%)
Presence of ultrasonography marker in the 2 nd trimester	00	24 (7.1%)	03 (1.2%)
Culture media failure	00	02 (0.6%)	04 (1.5%)
Antecedent aneuploidy	02 (1.7%)	05 (1.5%)	00
Parents bearing chromosopathy	01 (0.9%)	02 (0.6%)	00
Anxiety	00	01 (0.3%)	00
Anterior child with malformation	00	01 (0.3%)	00
Mosaicism in amniotic fluid	00	00	01 (0.4%)

CVS, chorionic villus sampling; NT, nuchal translucency.

Table 2 – Results of fetal karyotypes of the cases submitted to diagnostic invasive procedures.

Procedure/karyotype	CVS n (%)	Amniocentesis n (%)	Cordocentesis n (%)	Total n (%)
Normal	77 (68.2%)	214 (63%)	187 (67.3%)	478 (67%)
13 trisomy	03 (2.6%)	12 (3.5%)	12 (4.6%)	27 (3.8%)
18 trisomy	09 (8.0%)	30 (8.8%)	19 (7.3%)	58 (8.1%)
21 trisomy	10 (8.8%)	20 (5.9%)	14 (5.4%)	44 (6.2%)
X monosomy	06 (5.3%)	24 (7.1%)	04 (6.2%)	34 (4.8%)
Triploidy	0	04 (1.2%)	01 (0.4%)	05 (0.7%)
Other aneuploidy	01 (0.9%)	08 (2.3%)	10 (3.8%)	19 (2.7%)
Culture media failure	07 (6.2%)	28 (8.2%)	13 (5.0%)	48 (6.7%)

CVS, chorionic villus sampling.

Table 3 – Results of pregnant women who underwent invasive procedures for fetal karyotype study

Procedure/result	CVS 113 n (%)	Amniocentesis 340 n (%)	Cordocentesis 260 n (%)	Total 713 n (%)
Total intrauterine death	39 (34.5%)	125 (36.8%)	67 (25.8%)	231(32.4%)
Total intrauterine death associated with fetal malformation or abnormal karyotype	39 (34.5%)	124 (36.4%)	67 (25.8%)	230 (32.2%)
Legally induced abortion	01 (0.9%)	04 (1.2%)	03 (1.2%)	8 (1.1%)
Neonatal death	03 (2.6%)	36 (10.6%)	52 (20%)	91 (12.8%)
Born alive	71 (62.9%)	179 (52.6%)	141 (54.2%)	391 (54.8%)

Total intrauterine death, the sum of intrauterine deaths associated with fetal malformation or abnormal karyotype and legally induced abortion; total intrauterine death associated with fetal malformation or abnormal karyotype, total intrauterine deaths with malformation or abnormal fetal karyotype including legally induced abortions of altered fetuses; CVS, chorionic villus sampling.

Table 4 – Description of malformation by fetal system of the cases evolving to intrauterine death

Procedure/fetal malformation	CVS	Amniocentesis	Cordocentesis	Total
Hidropsy	10	23	9	42
Nervous system	3	17	15	35
Pulmonary	0	2	2	4
Heart	3	5	4	12
Digestive system	0	1	1	2
Abdominal wall defect	1	4	3	8
Urinary system	2	3	4	9
Skeletal dysplasia	1	7	3	11
Total	20	62	41	123

CVS, chorionic villus sampling.

to factors such as the non-existence of public policy for screening chromosomal abnormalities, difficulty of access to diagnostic examination services, patients fearful of adverse results after performing the procedures, and impossibility or limitation to treatment after certain diagnoses. In addition, this study was performed in a tertiary center where cases of abnormalities are referred to, many times in an advanced stage of pregnancy. Maternal age 35 years or over was, in the past, the main indication

for invasive procedures in developed countries. Presently, this indication has been substituted by the result of individual risk on screening tests in the first trimester by nuchal translucency measurements, sometimes combined with biochemical screening (free β -HCG and PAPP-A). In the present study, maternal age of 35 years or over represented 10.2% of indications and ultrasonography screening from the first trimester, is described by the increased translucency in 13.5%, describes the second and

the third commonest indications respectively. The other indications observed (antecedent aneuploidies, parents bearing chromosomopathies, and anterior child bearing abnormalities) presented frequencies similar to the medical literature^{23,24}.

In this study, it was also possible to observe a difference in the number of chromosomal abnormalities found (27.1%), a very high rate compared to other studies, which presented chromosomal abnormality rates up to 14%^{8,14,22}. This rate cannot be extended to the general population, since this is a reference service where patients are referred to for possible diagnosis and counseling.

Another difference observed is the culture media failure rate of 6.7% (48/713). The medical literature reports rates lower than 1.0%^{14,15,25,26}. This rate may be related to technical collection problems, as this is a teaching hospital, but it also indicates the need to review laboratory routines. Among the cases of culture media failure, in only six cases was a new puncture performed. Five cases of intrauterine death were observed among those cases of culture media failure, occurring in the time interval between the puncture and the return of fetal karyotype results. In 41 cases, the failure occurred in analyzing fetal karyotype in amniotic fluid or cordocentesis. The advanced gestational age in these procedures, approximately 20 weeks for amniocentesis and 27 weeks for cordocentesis, a period in which pregnant women initially feel fetal movements, may have influenced the decision to not perform a new puncture, for fear of a greater loss in advanced gestational age.

Adverse results, such as miscarriage, stillborn, and neonatal death also presented high rates when compared to rates in other studies: 4.9%, 25.7%, and 13% respectively. This was probably due to the presence of a great proportion of fetuses bearing chromosomal abnormalities and anomalies, besides the non-existence of a law for induced abortion in case of malformed fetuses. In 11 European countries, induced abortion by fetal indication is allowed regardless of the gestational age²⁷. In medical literature, miscarriage rates have been described from 0.35% to 2.58%; of stillborns, from 0.35% to 1.0%; and of neonatal death, from 0% to 8.33%^{14,17,21,28-30}. Antsaklis et al.³⁰, in fetuses presenting ultrasonography changes who underwent cordocentesis, reported 15.5% of intrauterine deaths and 8.33% neonatal deaths. These rates are lower than those observed in the present study, even though only fetuses bearing ultrasonography changes were included, probably demonstrating the severe condition in which such infants are referred to the service.

Knowledge of fetal karyotype has allowed counseling to pregnant women. In some cases, pregnant women were allowed to make decisions they deemed more adequate for their gestation, opting for legally induced abortion, obtained by preliminary order in cases of chromosomal

abnormalities incompatible with extra-uterine life. For pregnant women whose fetuses had severe abnormalities incompatible with life, chromosomal changes, or when they presented the need for psychological support, psychological counseling had been offered. The knowledge of fetal karyotype also allowed for appropriate delivery planning.

In the study, a patient who underwent amniocentesis with 16 weeks and five days of pregnancy died. The indication for performing the procedure was the presence of omphalocele and fetal cardiopathy. The karyotype result was 46, XY. The patient was hospitalized after 15 days, and discharged after a week of antibiotic therapy for pneumonia. Death occurred 22 days after the amniocentesis procedure. The maternal death was probably not related to the invasive procedure, as the pregnant woman had sickle cell anemia and had been on in other occasions during this pregnancy due to algic crisis, needing blood transfusion. Deaths related to invasive procedures take place after maternal sepsis, starting up to 30 hours after the procedure³¹; this fact does not appear to be related to this patient.

Among pregnant women with normal fetal karyotype and no structural changes, the gestational results were superior, presenting a survival rate of 99% (95/96) among all of the cases submitted to invasive procedures.

CONCLUSION

Fetal karyotype is an important diagnostic examination that should be offered to all patients after genetic counseling and screening test. This invasive procedure presents a risk of pregnancy loss; however, the present study demonstrates that the majority of fetal losses was related to a subjacent fetal condition (presence of fetal abnormalities and aneuploidies). Only one case evolved to intrauterine death with no fetal abnormality or abnormal karyotype. HCFMUSP still considers malformation and abnormalities as the main indication for fetal karyotype research, and such cases are referred to in advanced gestational age, which may be responsible for the higher number of cordocentesis (36.4%) for evaluation of fetal karyotype.

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