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Research Article

Prenatal diagnosis of fetal chromosomal abnormalities: Report of an 18-year experience in a Brazilian public hospital

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Abstract

The study of the fetal karyotype became an important tool for the fetal diagnosis of genetic diseases in the 1970s. Although application of this test has remained very restricted in Brazil, we had 905 referrals for prenatal fetal karyotyping between 1989 and 2007. In 879 cases, a fetal karyotype was obtained. We detected 74 abnormal karyotypes (8.4%), the majority being found when the prior indication was fetal malformation. When obtaining amniotic fluid or chorionic villus samples was difficult, alternative fetal materials (urine, cystic hygroma, cystic lung, intreperitoneal and cerebrospinal fluids) were collected and we had success in obtaining karyotypes in all 13 cases. Although, the option of terminating abnormal pregnancies does not legally exist in Brazil, the information gained in assessing the prognosis of on-going pregnancies or estimating recurrence risks justifies prenatal diagnosis of chromosome abnormalities. We conclude that, in keeping with the policy in most other countries, prenatal cytogenetic analysis is strongly recommended in high-risk pregnancies for fetal abnormalities. However, the unique aspect of this type of study is not its rarity in world terms, but its rarity in Brazil. This argues that Brazilian health policy on prenatal diagnosis requires reforming to make it much more widely available within the public health care sector.

Key words: prenatal diagnosis, chromosomal abnormalities, fetal malformations.

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Introduction

During the last decades the study of fetal karyotypes has become a very important tool for genetic counseling on recurrence risk and/or fetal chromosome diagnosis of at-risk pregnancies (Magalhães, 2001). Invasive prenatal diagnosis continues to be the standard method for searching for chromosomal aneuploidies or other genetic diseases (Bui, 2007). Prenatal diagnosis of cytogenetic abnormalities is now widely recognized as a reliable method with an acceptable risk for couples at high risk of giving birth to a child with clinically significant chromosome abnormalities (Caron *et al.*, 1999). Despite the fact that in Brazil amniocentesis and CVS were first introduced by Nazareth *et al.* (1981) and Gollop *et al.* (1988) respectively, there is still no public health care policy for application of cytogenetic prenatal diagnosis. As in other developing countries, this test is mostly confined to expensive private clinics, which means that it is rarely available for the great majority of pregnant women who depend on public medical services.

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Nevertheless, we have been offering this test in our public hospital since 1989. Prenatal diagnosis is a very restricted test in Brazil, mainly because induced abortion, even indicated by fetal genetic disease, is not legally allowed. Despite this, we have had 905 referrals for fetal karyotyping since it was first offered by our clinic in 1989. In the first four years, we had an average of 80 cases/year and this number decreased in the following ten years to 45 cases/year. In the last four years this number decreased even further, to 35 cases/year. This will be discussed later.

Even with the development of modern techniques, cell culture failure remains one of the main obstacles to be overcome. In order to improve the chance of getting a karyotype result, alternative fetal samples, such as urine or cystic hygroma fluid were used for chromosome analysis when malformations were found in the fetus and availability of conventional tissues was limited. The purposes of this study were: 1) to describe the most frequent indications for karyotyping the fetus in our socio-economic conditions; 2) to estimate the frequency of the most common prenatal chromosome abnormalities in patients from the Hospital de Clinicas de Porto Alegre; 3) to assess the cytogenetic results obtained with alternative tissue samples compared to amniocytes and chorionic villi.

Materials and Methods

Cytogenetic findings were retrospectively reviewed from 1989 to 2007 in 905 pregnant women, with a mean maternal age of 32.7 years, and mean gestational age of 22.7 weeks. Those women underwent prenatal cytogenetic evaluation only after a genetic counseling session, which means that risks, methods and indications were explained to the family. All samples were collected by a single gynecologist. The method used for sample collection was transabdominal punction guided by ultrasound. Samples were obtained for all patients, even in cases of lack of amniotic fluid, when alternative fluids were collected. Amniotic fluid, or any other fetal sample collected, were cultivated in long-term cell cultures, with Amniomax medium, at 37 °C in CO_2 incubator. Cordocentesis followed the standard blood culture that means, short-term culture (72 h) at 37 °C, and no requirement for a CO_2 incubator. We used standard Giemsa-banding staining technique for all chromosome analyses.

Results

The most frequent indications for prenatal cytogenetic diagnosis were advanced maternal age (with an average of 39.9 years old and mean gestational age of 18.7 weeks), abnormal findings on fetal ultrasound, a previous child with chromosomal abnormalities, and increased nuchal translucency (Table 1). Despite advanced maternal age being the most frequent indication for prenatal diagnosis, the majority of aberrant karyotypes were found when the indication was a fetal malformation detected by ultrasound. On the other hand, although the history of a previous child with Down syndrome was a relatively frequent indication, we did not find any positive cases in this group.

From the 905 prenatal cytogenetic analysis performed, we failed to obtain results in 26 (2.8%). Among the 879 karyotypes obtained, 74 (8.4%) were abnormal. (Table 1). Numerical abnormalities were found in 64 cases (7.3%), and structural aberrations in 10 cases (1.1%). The majority of numerical chromosomal abnormalities were autosomal trisomies. Trisomy 21 was the most frequent (28; 3.2%), and the second most frequent was trisomy 18 (24; 2.7%). Interestingly, trisomy 18 was almost entirely restricted to the group of "fetal abnormalities detected by ultrasound" and none was detected in the "increased nuchal translucency" group ($p < 0.001$). On the other hand, the difference in the frequencies of trisomy 21 between these two types of ultrasound prescreening was not statistically significant ($p = 0.096$). Trisomy 13 was found in six cases (0.7%), monosomy X in one (0.6%) and one case showed triploidy. Among structural chromosomal aberrations, translocations were the most frequent, and were detected in four out of the 879 cases analyzed (0.45%): reciprocal translocations in two cases and Robertsonian translocations in two others. Marker chromosomes were found in three cases, deletions in two cases and an inversion was present in one case.

In 13 cases alternative fluid samples were obtained (Table 2). The reasons for collecting alternative materials were lack of amniotic fluid in seven cases of kidney pathol-

Table 1 - Indications for invasive prenatal diagnosis and abnormal karyotypes.

Primary indication	Total number of cases (%)	Karyotypes obtained	Abnormal karyotypes (%)	Type of abnormalities (n)
Advanced maternal age	235(25.9)	227	13(5.7)	Trisomy 21 (10) Trisomy 18 (3)
Fetal malformation at ultrasound other than increased nuchal translucency	177(19.5)	169	38(22.5)	Trisomy 18 (19) Trisomy 21 (9) Trisomy 13 (4) 47,__,+mar (2) 45,X (1) Triploidy (1) 46,XX+13,der(13;14)(q10;q10) (1) 46,XY,del(18)(p?) (1)
Previous child with trisomy	125(13.8)	123	0	0
Increased nuchal translucency	65 (7.1)	63	9 (14.3)	Trisomy 21 (8) 47, XY,+mar(1)
Non immune Fetal hydrops	54 (5.9)	50	10 (20)	45,X (4) Trisomy 13(2) Trisomy 18(2) Trisomy 21(1) 46,XY,+14,der(14;21)(q10;q10)(1)
Others	249(27.5)	247	4 (1.6)	46,XX,+der(18)add(18)(p11)(1) 46,XX, t(15;16)(q21;p12)(1) 46,XX,inv(12)(q13q23)(1) 46,XY, t(7;10)(p21;q21)(1)
Total	905	879	74 (8.6%)	

Table 2 - Source of fetal material for karyotyping and success rate of cell cultures.

Fetus sample	Number of cases (n)	Culture success (n)	Success rate (%)	Gestational age in weeks (average)
Amniotic fluid	777	755	97.1	28.8
CVS	61	57	93.4	12.9
Cord blood	54	54	100	26.4
Alternative fluids[a]	13	13	100	31.2
Total	905	879	97.2	28.4

[a]Bladder (6), cystic hygroma (2), intraperitoneal (2), displastic kidney (1), cystic lung (1), cerebrospinal (1) fluids.

ogies, therapeutic drainage to facilitate delivery in six cases due to ascitis (n = 2), abdominal cyst (n = 2), pulmonary cyst (n = 1) and hydrocephaly (n = 1). The gestational age varied from 18th to 36th weeks with a mean age of 27.3 weeks. We had success in culturing these materials and in obtaining karyotypes in all cases (Table 2).

Discussion

Prenatal diagnosis has become a major aid to genetic counseling and for this, several important areas of technology have evolved, especially cytogenetic prenatal diagnosis, using analysis of cultured cells from the amniotic fluid at mid-trimester. Because of its high reliability and safety record with the lowest fetal loss and embryonic damage, amniocentesis has become the most common practice for prenatal diagnosis (Park *et al.*, 2001). However, CVS (chorionic villus sample) has gained popularity as a successful first trimester prenatal diagnostic technique since the mid 1980s (Brambati *et al.*, 1998), probably because of the advantage of establishing a diagnosis some weeks earlier in the pregnancy. Cordocentesis is a procedure used to obtain a sample from fetal blood directly from the umbilical cord in cases where amniocentesis is not possible or is used to give a quick result only in high-risk cases since procedure related pregnancy loss is high (Costa *et al.*, 1998).

Prenatal cytogenetic diagnosis using the above techniques was established in many countries, including Brazil (Gollop *et al.*, 1993; Pinto Jr, 2002), and has been performed for more than 18 years at the Hospital de Clínicas de Porto Alegre. During this period, the number of cytogenetic analyses has decreased by almost 50% per year in the Hospital and this can be explained by two facts: the introduction of nuchal translucency (NT) as a reliable screening method, and in the last four years medical insurance has provided payment for this exam, making it more accessible for the population. We would question whether NT alone is reliable to detect all forms of cytogenetic abnormality, since no cases of trisomy 18 were found in our NT sample (n = 65). On the other hand, when other forms of fetal abnormality detected by ultrasound were considered, then a frequency of trisomy 18 emerged which was even higher than trisomy 21 within this group. Intriguingly, our results suggest that prior diagnosis of fetal malformations using ultrasound is particularly efficacious for detecting trisomy 21 with the nuchal translucency test and, for trisomy 18, when other types of malformation are detected. However, Cheng *et al.* (2003) detected five cases of trisomy 18 among 171 instances of increased NT. This discrepancy might be due to our small size sample. Anyway, our results indicate that although ultrasound for nuchal translucency is strongly advised, any ultrasound prescreening should not be restricted to nuchal translucency, but should include also more generalized types of malformation, such as heart abnormalities, which are claimed to be present in almost all trisomy 18 fetuses. However, we feel that NT measurement used as a routine screening has decreased the number of referrals due to advanced maternal age, which has a low specificity, and has increased relatively the number of referrals for fetal abnormalities with a higher specificity. However, it has to be realized that tests such as nuchal translucency are not replacements for cytogenetic analysis, but provide strong indications for performing cytogenetic analysis in abnormal cases. The same arguments apply to serum screening in pregnant women. In some countries, such as the United Kingdom, increased maternal age is no longer applied as the sole referral indication for chromosome prenatal diagnosis; it is the combination of maternal age, serum screening and nuchal translucency and detection of other abnormalities by ultrasound which determines the validity of performing subsequent expensive cytogenetic analysis. However, all this is predicated on having all methods supported under the public health care system.

In a preliminary genetic counseling session, the approaches, methods and correct indications, are discussed with the family. In our sample, the history of a previous child with Down syndrome is the third more frequent indication. Although the risk of a recurrent trisomy is well established (Warburton *et al*, 2004), the risk is low and, not surprisingly, we did not find any recurrent case. Considering the current economic limitations to offer prenatal tests in our country, we propose that higher priority for the indication of prenatal diagnosis should be given to pregnancies where a malformation is detected on ultrasound scan than for couples who had a previous Down child, unless Down syndrome was caused by a Robertsonian translocation carried by one of the parents. This latter also assumes that post natal cytogenetic screening of all Down patients and, where necessary, their parents has occurred

already to identify those families with a high recurrence risk due to one of the parents being a carrier of a translocation involving chromosome 21. It is such families that will derive the most benefit from prenatal diagnosis. With such a directed policy we, and other centers, would be able to provide more opportunity for poor families with higher risks for fetal abnormalities to be assisted by prenatal diagnosis within the public health care system in Brazil.

The results of fetal cytogenetic abnormalities in our study are similar to those reported in the literature (Caron *et al.*, 1999; Carothers *et al.*, 1999; Quintana *et al.*, 1999). Several studies have shown that Down syndrome is the most common and clinically significant cytogenetic abnormalities detected in prenatal cytogenetic studies (Mathews *et al.*, 1992; Carothers *et al.*, 1999), followed by Edwards Syndrome (Song *et al.*, 1997; Han *et al.*, 2000). This was also found to be the case in our own series. The frequency of chromosomal abnormalities in the general population is estimated to be 0.5% of live births, but the frequency within the high-risk population is higher (around 5%, as observed in newborns with malformation by Nazer *et al.*, 2003, in Chile). The frequency of chromosomal abnormalities in our sample was even higher (8.5%) than other studies (Park *et al.*, 2001), probably because our Medical Genetic Service, as a reference center, receives patients who have been screened already by physicians in other Centers (without Genetic Services available) and are, therefore, more prone to having a chromosomal abnormality due to ultrasound alterations or familial history.

Karyotyping unconventional fetal samples, when it is difficult to obtain the traditional ones, is not a very common approach in most laboratories (Donnenfeld *et al.*, 2001; Gole *et al.*, 1997). We used this alternative when necessary and achieved a 100% success rate on an admittedly limited sample of 13 cases; however, the success rate is higher than that observed in other studies (Teoh *et al.*, 1996; Donnenfeld *et al*., 2001).

Although, the option of terminating genetically abnormal pregnancies does not legally exist in Brazil, the information gained in assessing the prognosis of on-going pregnancies or estimating recurrence risks for future family planning justifies prenatal diagnosis of chromosome abnormalities. In our sample the three most frequent indications were advanced maternal age, fetal malformation at ultrasound and a previous child with trisomy. However, the majority of aberrant karyotypes were found in the group with a fetal malformation detected by ultrasound and, as argued above, this opens up the possibility of triaging the initial referral group and being more efficient in deriving the maximum benefit to the maximum number of patients under limited resources.

Although, the benefit of using "alternative" fetal samples for karyotyping is marginal in terms of numbers this approach can provide a karyotype result to high-risk families in situations where it has proven impossible to derive traditional tissues for analysis, even in advanced gestational age.

In general the analysis of our data supports the contention that the wide practice performed in many other countries of prenatal cytogenetic analysis being made available to the whole population and performed routinely in high-risk pregnancies, should also take place in Brazil within the public health care sector and not be almost entirely confined to the private care sector, as at present. However, a solid public health care policy for prenatal diagnosis needs to be established in which the distribution of facilities and reasonable coverage of expenditures has to be evaluated.

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