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Original article

Prenatal diagnosis of chromosome disorders in Tunisian population[#]

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Abstract – Cytogenetic prenatal diagnosis (PND) is under national health program in most developed countries, while it concerns a small part of population at risk in developing countries. Finance is common reason of absence of PND development, but socio-cultural believes play an important role in Arab Muslim countries. In this paper we report results of 3110 fetal karyotypes carried out in a Tunisian population, by cultured amniocytes analysis. It is the largest report in a Muslim Arab country in our Knowledge. Abnormal karyotypes rate was 4.18% classified in two groups: bad prognosis (3.05%) and good prognosis (1.13%). Common amniocentesis indication was maternal age. The highest predictive value was observed in balanced karyotype and fetal ultrasound findings indications. Maternal serum markers were not commonly used for trisomy 21 screening. Pregnancy termination that is permitted by legal and religious authorities was accepted by 94,74% parents. Information about PND outcomes was given by genetic counselling prior to fetal sampling, pregnancy interruption was discussed with parents at cytogenetic result announcement. The authors conclude that in order to prevent mental and physical handicap related to cytogenetic disorders we have to promote PND by education for population, genetic counselling and fetal ultrasound screening ; all three methods available in Tunisia. © 2001 Éditions scientifiques et médicales Elsevier SAS

chromosomes abnormalities / prenatal diagnosis / amniocentesis / arab muslim / pregnancy termination

1. Introduction

Prenatal diagnosis (PND) presently constitutes a large activity of preventive care in developed countries. A growing number of methods for the in-utero diagnosis of fetal disease became available during recent decades in ultrasound technology and laboratory methods. Exclusion of fetal aneuploïdy in pregnancies of advanced maternal age is still by far the most common reason for an invasive procedure: amniocentesis and chorionic villous sampling. Unfortunately prenatal diagnosis of chromosome disorders leads to pregnancy interruption for lack of treatment.

In Tunisia that is an Arab Muslim country abortion is legally allowed when medically indicated. Prenatal diagnosis of chromosome anomalies, metabolic disorders and several mendelian diseases started in Tunisian laboratories in 1989 and became available in routine few years later. In this paper we limit our report to a study of prenatal cytogenetic diagnosis by amniotic cells analysis carried out in our laboratory during last four years.

Our objectives are to present a series of prenatal diagnosis in a Muslim, Arab country; to discuss the acceptance of pregnancy termination by parents; to compare cytogenetic results and indication distribution with those established in other countries and to propose how to promote PND of chromosome disorders in a developing country.

2. Materials and methods

2.1. Materials

We have standardised the technique of prenatal cytogenetic analysis in the laboratory on September

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Table I. Distribution of amniocentesis indications.

Indications	Analyses	
	N	%
Advanced maternal age > 35 years	2023	65.05%
Previous child with congenital malformations and/or mental retardation	366	11.76%
Chromosomal diseases in close family	213	6.85%
Parental balanced karyotype	30	0.96%
Ultrasound scan abnormalities	256	8.23%
Positive maternal serum markers	60	1.95 %
Others	162	5.21%
Total	3110	100.00%

1996, so we will present results of fetal karyotypes carried out from this date until September 2000. During this period 3110 fetal karyotypes were analysed from cultured amniotic cells for pregnancies at risk, indications are reported on *table I*.

The patients were referred by obstetricians from private health institutions (29%) and public hospitals (71%). For each patient 20 to 30 ml of fresh amniotic fluid were obtained by transabdominal amniocentesis between the 14^{th} and 35^{th} weeks gestation, depending on karyotype indication. Gestational age was calculated from last menstrual period for those with regular cycles and from ultrasound measurement of the crown-rump length for the others. The mean gestational age for amniocentesis in our series was 17 weeks. The other invasive procedures as chorionic villous and foetal blood sampling were excluded from the study in order to have homogeneous material.

2.2. Cytogenetic analysis

Amniocytes collected after amniotic fluid centrifugation were cultured in 3 different flasks using standard technique [1]. Cultures were harvested when three to four colonies were confluent,12 to 15 days after seeding. Chromosomes were systematically banded, using R–bands by heating and Giemsa (RHG). In some cases analysis was completed by G-banding and Nor technique. For each karyotype 15 to 20 mitoses were studied, and in case of mosaïcism up to 30 mitoses were analysed.

Parental blood samples when referred were stimulated with PHA and cultured for 72 hours before harvest, chromosomes were banded using RHG technique [1, 2].

In 64 cases we needed a second sampling because of contamination in 78% and culture failure in remaining cases.

2.3. Clinical information methods

Previous to amniocentesis, information was given to the patient at genetic department clinics about significance of fetal karyotype abnormalities and about prenatal diagnosis outcomes including amniocentesis risk and pregnancy termination indication. Sometimes information was given at the time of sample referral. When abnormal fetal karyotype belonged to the bad prognosis group that we will define below, pregnancy interruption was discussed with parents. In case of acceptance, termination was performed by obstetrician in charge. We classified the chromosomes abnormalities in terms of bad prognosis and good prognosis and defined two groups. The bad one included autosomal aneuploïdies involving trisomies and unbalanced structural abnormalities ; polyploidy ; sex chromosome aneuploïdies 45,X ; 47,XXY and poly X with total chromosomes number upper than 47. The good prognosis group included the other sex chromosomes abnormalities (47,XXX ; 47,XYY ; structural anomalies) and balanced chromosome rearrangements with normal ultrasound scan. The extra chromosome markers were classified depending on if their were inherited or de novo, on fetal ultrasound scan and on literature description and reports.

3. Results

A total of 3110 fluid samples were analysed using conventional cytogenetic with R-banded chromosomes obtained by amniotic cells culture. We detected 130 abnormal fetal karyotypes giving an incidence of 4.18%. Abnormalities consisted of autosomal aneuploïdies with trisomy 21, 18, 13, sex chromosome abnormalities : 45,X; 47,XXY ; 47,XXX ; 47,XYY ; 48,XXXY and structural abnormalities that included unbalanced and balanced chromosomes and finally

Table II. Cytogenetic Results and prognosis of 130 abnormal fetal karyotypes.

Cytogenetic Anomaly	Homogeneous	Mosaic	Total	Prognosis		Induced Abortion
47,+21	52		52	BP		50
47,+18	12		12	BP		12
47,+13	5		5	BP		5
45,X	4	1	5	BP	GP	4
47,XXX	4		4		GP	1
47,XXY	5		5	BP	GP	3
46,XY/47,XYY		1	1	GP		-
48,XXXY	1		1	BP		1
46, XX/47,XX+14		1	1	BP		1
47,+extra-chromosome	3	1	4	BP	GP	1
69 XXY	3		3	BP		3
46,XX/47,XX+ 20		1	1		GP	-
46,XiXq/47,XX + i Xq		1	1		GP	-
Unbalanced Structural Anomaly						
46,t(11,13)(q24;q14) +13q	1		1	BP		1
46, t(9,14)(p12;q32)+der14	1		1	BP		1
46,t(13,21) +21q	1		1	BP		1
46,t(14,21) +21q	2		2	BP		2
46,t(21,21) +21q	1		1	BP		1
46,t(1,3)(q31,qter) +der 3	1		1	BP		1
46,XYt(1,11)(q5qter)p14pter der11	1		1	BP		1
46, r (22)	2		2	BP		2
Sub-Total	*99*	*6*	*105*	*95*	*10*	*91*
Balanced Structural Anomaly						
46,XY t(11,22)(p11;p12)	1		1	GP		
46,t (18,22) (q21;q12)	1		1	GP		
46, / 46, t(8,10) (q22; 25)		1	1	GP		
46, t (1,3) (p14; pter)	1		1	GP		
46, t (20,22) (qter ; q12)	1		1	GP		
46, t (7,14) (p12;q11)	1		1	GP		
46,t(17,15)(q23 ; qter)	1			GP		
46, t(6,14) (q15 ;p12)	1		1	GP		
46, t(11,3) (p21;q ter)		1	1	GP		
45, t (13,14)	7		7	GP		
45,t (14,21)	1		1	GP		
Chromosome Inversion	6		6	GP		
Undefined abnormal chromosome		2	2	GP		
Sub-Total	*21*	*4*	*25*	*25*		*0*
TOTAL	122	8	130	95	35	91

extra-chromosome markers. As previously indicated we were interested in the prognosis of the chromosomes abnormalities and classified the karyotypes in two groups. The total number of bad prognosis group (*table II*) was 95 giving a rate of 3.05%. The most frequent anomaly was trisomy 21 followed by trisomy 18, 13; X aneuploidy and different structural abnormalities. For all these cases pregnancy termination was discussed with parents and have been accepted by 90 couples. The group that we considered of good prognosis (1.13%) included abnormal sex chromosomes (except 45X, 47,XXY and 48, XXXY) and balanced structural abnormalities. Parental chromosomes study if not previously established was performed for cases with structural abnormality or extra-chromosome marker in order to precise inherited or de novo rearrangement. Mosaïcism was observed in 8 cases that represented 6.15% of abnormal karyotypes, all cases were considered of good prognosis except trisomy 14 [3]. An extra-chromosome marker was observed in 4 cases. Fetal ultrasound scan was proposed for all cases in this group and pregnancy was maintained according to normal scan (see *table II*).

4. Discussion

This study shows that during four years, cytogenetic PND was provided to 3110 Tunisian women after amniocentesis. The volume of examinations is

Table III. Annual increasing number of foetal karyotypes.

Period	Analysis
09/1996–09/1997	689
09/1997–09/1998	758
09/1998–09/1999	807
09/1999–09/2000	856
Total	3110

significantly smaller than reports in western and developed countries. But instead of its small number, the series constitutes to our knowledge the largest report in an Arab Muslim country. National health policy for PND is not established in Tunisia, but increasing annual number of prenatal karyotypes carried out in the laboratory (*table III*) indicates that PND is being gradually accepted in our community by patients and health professionals.

4.1. Fetal karyotype indication

Four principle reasons led to fetal cytogenetic analysis (*table I*) that is similar to European series at the beginning of PND [4–7]. Advanced maternal age was the common indication (62.5%). Amniocentesis is indicated for women aged 35 years old and over. Previous child or fetus with congenital malformations and or mental retardation was the second reason (11.76%). Because of lack of well-documented propositus dossier, fetal chromosome analysis was estimated necessary to prevent chromosome disorder recurrence. Maternal serum screening (MSS) that consisted of testing AFP, HCG and fE3 ; represented a rare indication for fetal karyotype in our series that is comparable to a Danish series studied between 1980 and 1993 [6] but different from other old studies [8], in France for example this percentage was 9.1% in 1994 [9]. However MSS percentage is slightly increasing in our series, 2.28% in 2000 while mean percentage was 1.95%. We think that few obstetricians prescribe the test and even they do, prescription is not systematic for all patients. Abnormal ultrasound findings indicated fetal karyotype in 8.23% of our series. Ultrasound findings consisted of two groups : morphological abnormalities and abnormal nuchal thickness. Fetal US screening seems more commonly used by obstetricians ; its contribution to indicate fetal karyotype is increasing (13.20% in 2000) ; but remains low comparing to most series [6, 9]. Foetal US findings involved the first trimester measurement of nuchal translucency thickness, cystic hygroma [10]; and the second trimester scan of fetal abnormalities [11].

4.2. Predictive value for fetal karyotype indication

Prenatal cytogenetic analysis detected 4.18% abnormal karyotypes and 3.05% were bad prognosis in our series. The highest predictive value was observed for balanced parental karyotype (*table IV*), 26.66% of fetal karyotypes were abnormal (16.66% were unbalanced) in this group, that is concordant with calculated risks in other report [12]. Ultrasonography offers a non-invasive means to improve the selection of pregnant women who may be candidate for invasive prenatal diagnosis, by evaluation of fetal anatomy for detection of structural anomalies and by utilising a series of markers that are more frequent in fetuses with abnormal karyotype [8, 13, 14]. Fetal ultrasound findings were good indicators for diagnosis and 9.01% of them detected chromosome abnormalities in our series. Femur length have been considered for long time as a significant predictive marker of chromosome anomaly, in particular trisomy 21. In two cases this marker allowed us to detect trisomy 21, but recent published literature suggests that it is not consistent predictive marker [15]. Actually nuchal translucency thickness measure is considered as a high predictive indicator of chromosome anomaly [16, 17].

Table IV. Predictive value of abnormal karyotypes for amniocentesis indications.

Indications	*Abnormal Karyotypes*	
	N	%
Advanced maternal age > 35 years	80	3.95%
Previous child with congenital malformations and/or mental retardation	6	1.64%
Chromosomal diseases in close family	5	2.35%
Parental balanced karyotype	8	26.66%
Ultrasound scan abnormalities	23	9.01%
Positive maternal serum markers	2	3.33%
Others	6	3.70%

As previously mentioned maternal test screening had rarely indicated fetal karyotype in our series indication was based on cut off value of 1/250; its predictive value was low (3.33%) comparing to literature reports. Additional to statistical bias and laboratory method, using European norms and cut-off value for Tunisian patients may be a source of error. Further studies have to demonstrate significance of the test in our population.

Finally advanced maternal age, the common reason for fetal karyotype was predictive of chromosome anomaly in 3.95%. In fact it allowed us to detect 61.53% of abnormal karyotypes diagnosed in our series. However combination of maternal age risk, nuchal translucency thickness value, that define a cut-off as reported by Nicholaïdes et al. [18], and Bahado et al. [19] will surely help to increase prenatal detection of chromosome disorders.

4.3. Pregnancy termination

Parents of 95 fetuses with abnormal karyotype belonging to bad prognosis (*table II*) were informed about phenotype expression of chromosome unbalance, physical and mental outcomes, then pregnancy termination availability was discussed with both parents; they had to make decision themselves about maintaining or terminating pregnancy. Finally 90 couples (94.74%) all Muslims opted for abortion (*table II*). Percentage of pregnancy termination acceptance in our series was much higher than reported in an Arab population [20, 21]. Three couples refused interruption, one of them because gestation was too advanced when result was given. For two remaining cases, parents decided to continue pregnancy with 'Klinefelter syndrome'. Pregnancies with a diagnosis of 47,XYY and 47,XXX were continued in absence of foetal ultrasound anomaly more often than pregnancies with Turner (45,X) and Klinefelter (47,XXY) syndromes. This difference may result from parental concerns of having a child with infertility [22] or because of association with physical or behavioural manifestations [23]. However for one fetus with 47,XXX the mother didn't accept to continue pregnancy because of behavioural manifestations risk.

Couples acceptance is related to quality of information received during genetic counselling, they were prepared to accept pregnancy termination as a possible event. Parents approbation of prenatal diagnosis and non-refusal of induced abortion were needed before taking fetal sample in most cases of our series.

5. Conclusion

In this study we demonstrate that cytogenetic prenatal diagnosis followed by termination of pregnancy for bad prognosis karyotypes was accepted and carried out in an Arab Muslim population. Pregnancy termination allowed by Tunisian law, was permitted by religious authorities and accepted by parents. In absence of national program for cytogenetic PND, fetal karyotype indication depends upon physician knowledge and patients education, information, conviction and financial means. Informed about high risk of genetic disorders, parents ask for prenatal diagnosis and approve pregnancy interruption. Approbation depends of course on personal convictions and not necessarily on religion group. Cytogenetic PND is then possible in Arab Muslim community. To promote this prevention activity with moderate cost we have to develop genetic counselling, population education and fetal ultrasound screening. All three actions are actually available in Tunisian health institutions and may play a role in physical and mental handicap prevention.

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