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RESULTS OF CYTOGENETIC ANALYSIS OF 521 AMNIOTIC FLUID CELL CULTURES (AMNIOCENTESES) PERFORMED IN IRAN

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ABSTRACT

The indications and results of cytogenetic analysis of 521 amniotic cell cultures performed at 13-16 weeks of gestation were evaluated in this study. 507 fetuses (97.3%) were cytogenetically normal, 14 (2.7%) had unbalanced karyotypes, and 2 fetuses were found to have major abnormalities, one with anencephaly detected by measurement of alpha -fetoprotein levels in amniotic fluid and ultrasonography, and the other with a full mutation at the FMR1 locus detected by molecular techniques. The unbalanced karyotypes included 2 cases each of trisomy 21, trisomy 18, triple X, 47 XXY and mosaicisms; and 4 cases of various chromosomal abnormalities.

225 tests were performed for women 35 years of age or over, 6 (2.4%) abnormal karyotypes were detected, showing a 6 - fold increase over the general population. This risk was even higher (2/23, 8.7%) among those parous aged >35 y who had a previous history of offspring with chromosomal aberrations.

5 of 32 (15.65%) fetuses whose parent was a carrier of a balanced chromosomal translocation or a small chromosomal marker (in one case), had unbalanced chromosomal aberrations.

There were 7 cases of spontaneous abortion within 4 weeks after amniocentesis, one of which was a case of 46 XY, t (14;21), +18. Excluding this case, the rate of abortion (6/521, 1.3%) was quite below the expected rate of 2.1% for spontaneous abortion in the 2nd trimester of pregnancy. Our data indicates that amniocentesis performed at 13-16 weeks is a safe, reliable procedure for detection of fetal chromosomal abnormalities in Iran, and we strongly recommend it for those parous at risk.

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INTRODUCTION

Aminocentesis is still one of the most widely used procedures for fetal sampling and prenatal diagnosis.[1] In 1903 Balantin proposed the suitability of amniotic fluid for evaluation of the fetal condition.[2] In 1954, sex chromatin was analyzed in amniotic fluid cells for sex determination.[3] Amniotic cells were cultured in 1965 by Steele and Berg.[4] Jacobson and Barter reported the first case of trisomy 21 in cultured amniocytes in 1966.[5]

Initially, and before the advent of sonographic technology, amniocentesis was performed blindly, limiting the procedure to 16-20 weeks of gestation and with a considerable risk of abortion. The development of sonography as an intrauterine visualization technique has decreased this risk and made earlier amniocentesis feasible. Early amniocentesis, 10-14 weeks from LMP, was suggested in 1987 [6-7] and is now being tested in various laboratories. From Nov. 1989 to March 1999, 793 prenatal tests have been performed in this center for the following purposes.

1 - Chromosomal abberation	559	cases
2 - Hemoglobinopathy (beta thalassemia)	190	cases
3 - Inborn errors of metabolism	30	cases
4 - Triple nucleotide repetition (Frag. X)	5	cases
5 - Muscular disorders (MDD, BMD, SMA 1& 2)	4	cases
6 - Skin lesion (xeroderma pigmentosum & Cockayne syndrome)	5	cases

The results of 521 amniotic fluid cell cultures for cytogenetic analysis are being reported. To the best of our knowledge, the first chromosomal study of amniotic fluid cells in Iran was performed in November 1989 at our center.

MATERIALS AND METHODS

During a 9 year period, 521 amniocenteses were performed successfully for prenatal diagnosis of chromosomal abnormalities. During a counseling session, a complete genetic history is taken and the couple are advised as to the procedure and possible risks. The couple are asked to sign an informed consent form.

About 10 - 20 mL of amniotic fluid is aspirated by an experienced gynecologist under continuous ultrasound guidance, preferably in 2 syringes which are numbered and cultured separately; in cases of mosaicism it is essential to compare the two samples for distinguishing between pseudo and true mosaicism. Amniotic fluid is centrifuged at 1500 rpm for 10 minutes.

The supernatant is sent for measurement of alpha-feto-protein levels. The sediment is cultured in Ham F-10 medium, supplemented with 20% fetal bovine serum, Ultroser G., L-glutamine, penicillin-streptomycin, fungizone, kanamycin and Hepes buffer. The cells are let to settle for 5-6 days and thereafter, the medium is changed twice weekly. All cultures are controlled daily under inverted microscopy until optimal cell growth is obtained. The cells are detached using Trypsin EDTA and harvested with serum based hypotonic solution and fixed in 3 washes of Carnoy's fixative. The slides are treated with trypsin for G-banding. At least 15 metaphase spreads are analyzed. The

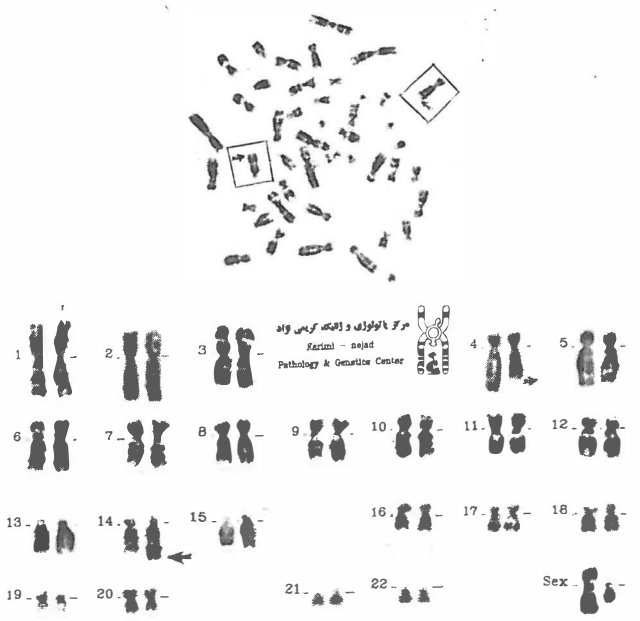

Fig. 1-a. A metaphase spread of amniotic cell karyotype (Am. No. 406) reveals 46 XY, t (4;14). The involved chromosomes are encircled. The arrows show the breakage points.
b. An arranged karyotype of the above spread.

Fig. 2-a. Spread of amniotic cell karyotype (Am. No. 271) shows 46 XY, t (14;21) + 18. The translocated (14;21) and 3 No.18 chromosomes are encircled.
b. The chromosomes of group D (13-15), E (16-18), G (21 - 22), and sex chromosomes are arranged.

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Table I. Results of 521 amniotic cell cultures.

Indication	Number	%	Normal karyotype	%	Balanced chromosomal abberation	%	Abnormal karyotype	%
Advanced maternal age >35	225	43	219	97.3	0	-	6	2.7
History of offspring with chromosomal abberation	154	29.5	153	99.35	0	-	1	0.65
History of offspring with chromosomal abberation	23	4.4	21	91.3	0	-	2	8.7
Balanced chromosome abberation in either of the parents	M. 22 32 F. 10	6. 14	13	40.6	14	43.8	5	15.6
Loss of offspring, pre- or postnatal death	40	7.7	40	100	0	-	0	-
Sex determination	11	2.1	11	100	0	-	0	-
Abnormal CVS karyotype	5	0.96	5	100	0	-	0	-
Miscellaneous	31	5.95	30	96.77	1	3.23	0	-
Total	521	100	492	94.4	15	2.9	14	2.7

above procedure takes approximately 10-14 days. In case of culture failure, a repeat amniocentesis is requested.

RESULTS

The indications for, and the results of cytogenetic analysis of 521 amniotic fluid cell cultures are shown in Table I. The largest number of referrals [225 (43%)] were mothers with advanced maternal age (>35y), 10% of whom also had a history of offspring with abnormalities such as mental or developmental retardation, hemoglobinopathy, metabolic disorder, Fragile X, etc. Six abnormal karyotypes (2.7%) were found as follows: 2 cases of 47 XXX, 1 of 47 XXY, 1 of trisomy 18, 1 of Down syndrome, and 1 case of aberration in chromosome 17.

The second largest group, 154, were those mothers with a history of previous offspring with the following chromosomal abnormalities: 145 (94%) with trisomy 21 (DS), 2 with trisomy 13 (PS), 2 with monosomy X (Turner), 1 with trisomy 18 (ES), and 3 with other abnormalities. The fetal karyotypes were normal except for 1 case (0.65%) with 47 XXY.

23 mothers were tested for advanced maternal age and a history of offspring with chromosomal aberrations, mainly Down syndrome. Two (8.7%) abnormal fetal karyotypes, 47 XXY and 46 XX/46 XX, mar 12, were detected.

The fourth group at highest risk were 32 couples with a balanced translocation, or a small marker in one case in either partner. 5/32 karyotypes (15.65%) showed a chromosomal abnormality, while of the remaining 27 cases, 13 were normal, and 14 were carriers of a balanced translocation, similar to their parent.

The 5th group is comprised of 40 mothers (7.7%) who had former experience of IUFD or perinatal death of offspring with or without congenital anomalies. Fetal sex determination was the major indication for 11 tests (2.17%), where the mother was known to be a carrier of an X-linked disorder (6 cases), or there had been a former child with congenital adrenal hyperplasia. 5 tests (0.95%) were requested for verification of an abnormal fetal karyotype detected in a CVS sample from another lab.

The last group is composed of 31 amniocenteses performed for miscellaneous reasons, such as high or low maternal serum alpha-fetoprotein levels, metabolic study of amniotic fluid cells or molecular study for CGG repeat at FMR1 locus, where chromosomal study was performed as an additional test. One fetus had anencephaly and another had a full fragile X mutation. One balanced t(4:14) was observed in the fetus of a woman being tested for β thalassemia who also had a history of first trimester abortions (Fig.1-a, b).

492 karyotypes (94.4%) appeared normal, 15 (2.9%) showed balanced translocations and 14 (2.7%) had an unbalanced chromosomal constitution. The abnormal karyotypes are shown in Table II. There were 2 cases of trisomy 21, two of tri 18, 2 of triple X, two of 47 XXY and

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Table II. Indication, karyotype of fetus, and parents.

Am. No.	Indication	Fetus	Mother	Father
12	AMA	47,XXX	-	-
107	F. bal. t(2,13)	46, XY, -13+der (13),t(2;13)(P23;q34)	Normal	bal. t(2;13)
227	AMA. 40y	46, XX, mar. 17	-	-
258	AMA. 39y	47, XXX	-	-
262	Ab. + Off with mar. 12	46, XX/46, XY, mar. 12	-	-
271	F. bal. t(14;21)	46, XY, t(14;21), +18	Normal	45, XY, t(14,21)
273	M. car. mar. chro.	47, XY, +21	47, XX + mar.	Normal
334	AMA. 42y	47, XY, +21	-	-
345	AMA. 39y + Off DS.	45, X/46, XX/47, XXX	-	-
433	AMA. 46y	47, XY, +18	-	-
462	F. bal. t(4;7)	46,XY, -4 + der(4), t(4;7) (q35; q32)	Normal	46,XY, bal. t(4,7)
479	AMA. 39y + Off DS.	47,XXY	-	-
483	AMA. 40y	47, XXY	-	-
548	M. bal. t(5,7)	46, XY, del (5p13:)	46, XX, bal. t(5.7)	Normal

Ab. = Abortion, Am. = Amniocentesis, AMA = Advanced maternal age, bal. t = balanced translocation, car. = carrier, DS = Down syndrome, F.= Father, mar. = marker, M = Mother, Off = Offspring

Table III. Mosaicism of AFC karyotype and outcome.

Am. No.	Tube	Plate	Results	Outcome
Am. 256	1 2	36 50	46, XX [27]/47, XX + 7[9] 46, XX	Normal female
Am. 262	1 2	23 32	46, XX [17]/46, XX, der 12[6] 46, XX	Abnormal genitalia
Am. 345	1 2 3	30 30 30	45,X/46, XX/47, XXX 45,X/46, XX/47, XXX 45,X/46, XX/47, XXX	Termination of pregnancy

2 mosaic patterns and 4 cases of various other chromosomal abnormalities, namely der 13, mar 17, der 4, and 5p- (Fig. 2-a, b).

Three cases of mosaicism were noted (Table III). Case 256 was referred for confirmation of 46 XY marker 17 reported in a CVS culture from another lab. Chromosomal study of AFC revealed a 47 XX + 7 pattern in 9 out of 36 analyzed plates from the first tube (Fig. 3) while all 50 analyzed spreads from the second tube revealed a normal 46 XX pattern. As this chromosomal finding was seen in only one tube, after consultation with expert colleagues we were inclined to consider it a pseudomosaicism. The baby girl is now 28 months old and doing well. The second case was a mosaicism of 46 XX/46 XX, mar 12, detected in 6 out of 23 spreads analyzed from the first tube while the 32 spreads from the second tube were all normal. The couple opted to terminate the pregnancy at 4.5 months; the physician declared ambiguous genitalia in the fetus, which could be questionable without performing a postmortem study.

The third case was a 45 X/46 XX/47 XXX mosaicism, observed in all three cultured tubes, and considered a true mosaicism.

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Overall, 16 of 521 cases (3%) were candidates for pregnancy termination. All remaining parents have been contacted 7 months to 3 years after their tests and were questioned as to the health and sex of their offspring. All replies show full correlation between outcome of pregnancies and the test results as to sex. Seven pregnancies were aborted spontaneously within 4 weeks after amniocentesis, 1 at 14 weeks, 5 at 15-16 weeks and 1 at 22 weeks. 6 had normal karyotypes and one had trisomy 18 (46 XY, t (14,21), +18).

DISCUSSION

Amniocentesis is now accepted as a safe and reliable method for cytogenetic examination of the fetus.[1] A collaborative study of 8000 amniocenteses estimated rates of 1.25% for culture failure, 1% for abortion and 2.4% for chromosomal abnormality, while our data for the above are 0.76%, 1.15% and 2.7%, respectively, indicating that amniocentesis and cytogenetic analysis of amniotic fluid in Iran is not associated with any increased risk. 12 cultures (2.4%) required repeat amniocentesis due to failure on the first attempt. Four failures (0.76%) were due to late gestational age (28 weeks), inadequate sample, bloody sample, and heavy contamination, which are not included in Table I.

Fourteen karyotypes (2.7%) were abnormal, including one case of true mosaicism. The abnormal karyotypes were found in fetuses of mothers who had been tested for one of 4 reasons: 6/225 (2.7%) for advanced maternal age, 1/154 (0.65%) for a history of offspring with chromosomal abnormality, 2/23 (8.7%) for advanced maternal age and offspring with chromosomal aberrations, and 5/33 (15.1%) for being a carrier of balanced chromosomal abnormality in either parent. Unbalanced chromosomal abnormalities were not detected in any of the other indication groups.

The rates of 2.7% in the first and 8.7% in the 3rd groups indicate a 6 and 14.5 fold increase in risk over the general population. The group at highest risk was those couples in whom either parent had a balanced translocation. 5 of 33 (15.65%) of their fetuses had unbalanced karyotypes, confirming the increased risk of a non-disjunction resulting subsequent to a chromosomal rearrangement in the individual, with a balanced chromosomal aberration, a phenomenon first suggested by Turpin & Lejeune in 1965.[9] There were 2 cases of 47 XXY among male fetuses, which is much more than the expected 1/450 live male births. Spontaneous abortion occurred in 7 out of 521 cases, including one abortus with trisomy 18. Seven out of 52 pregnancies is quite lower than the 2.1% spontaneous abortion rate given for all pregnancies with documentation of fetal viability by 10 week ultrasounds.[10] Unfortunately, we have no information on the outcome of those pregnancies in which amniocentesis may have been attempted, but amniotic fluid not obtained. Only one abortion occurred in the 134 cases of early amniocenteses (0.74%) at 12-14 weeks of pregnancy.

In conclusion, excluding the 16 cases of abnormal fetuses and 6 abortions, 499 parous women proceeded through their pregnancies safely and with peace of mind, till delivery. We highly recommend 13-16 weeks–optimal 14 weeks–amniocentesis, for chromosomal study in the following pregnancies:

1 - Either parent being a carrier of balanced or unbalanced chromosomal aberration.

2 - Advanced maternal age >35y with or without history of having affected offspring.

3 - History of offspring with chromosomal aberration.

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