CYTOGENETIC ANALYSES OF SPONTANEOUS ABORTION MATERIALS REVEALED FREQUENTLY NOTED AND RARELY NOTED NUMERICAL ABNORMALITIES: REPORTING OF THE TEN YEARS EXPERIENCE

Spontan Abort Materyallerinin Sitogenetik Analizinde Sık ve Nadir Görülen Sayısal Anomaliler: 10 yıllık Deneyimin Sunumu

***MERAL YİRMİBES KARAOGU*[1], GULSUM KAYHAN[1],ELİF PALA [1], AYSEGUL OZTURK KAYMAK[1], AYDAN BİRİ[2]**

[1]Gazi Üniversitesi Tıp Fakültesi, Tıbbi Genetik Ana Bilim Dalı, Ankara, Türkiye
[2]Gazi Üniversitesi Tıp Fakültesi, Kadın Hastalıkları Ve Doğum Ana Bilim Dalı, Ankara, Türkiye
[1] Department Of Medical Genetics, Gazi University Faculty Of Medicine, Ankara, Turkey
[2]Department Of Obstetrics And Gynecology, Gazi University Faculty Of Medicine, Ankara, Turkey

ÖZET

Amaç: Spontan abort materyallerinde belirlenen kromozomal anomaliler on yıllık döneme aittir. Belirlenen anomalilerin özellikleri, anne yaşı ve gebelik kayıp sayılarının detayları tartışılmaktadır.
Gereç ve yöntemler: İki ayrı flaskta primer doku kültürü kurulmuş ve sitogenetik analizler Giemza bantlama sonrası yapılmıştır..
Bulgular: Genetik Tanı Merkezimize sitogenetik analiz amacıyla 1046 abort materyali refere edilmiş ve bunlardan 1016'sında doku kültürü başarılı olmuştur (%97.1). İkiyüzoniki olguda (%20.1) kromozomal anomali belirlenmiş, bunlardan otozomal trizomi saptanan 117 (%55.2) olgunun 57'sinde ise anne yaşının 35'den büyük olduğu gözlenmiştir. Literatür ile uyumlu olarak en sık trizomi 16 rapor edilmiştir (22 olguda). İkili ve üçlü anöploidiler 12 olguda (%5.7) analiz edilirken, sırasıyla 27 (%12.7) olguda ve 40 (%18.9) olguda ise monozomi X ve poliploidi belirlenmiştir. Altı olguda ise hem poliploidi hem de anöploidi saptanmış; bunların içinde triploidiye daha fazla oranda trizomi 7'nin eşlik ettiği görülmüştür Tekrarlayan gebelik kaybı olan sekiz olgunun refere edilen abort materyalerinde tekrarlayan trizomiler ve poliploidiler saptanmıştır. Yapısal anomali 8 olguda görülmüş, bunlardan dört tanesinin dengesiz kromozomal yapıda olduğu belirlenmiştir. Normal karyotiplerin içinde 94 tanesi XY iken, 710 olguda abort materyalinin muhtemelen anne desidual doku ile kontamine olmasına bağlı olarak XX karyotipi rapor edilmiştir.
Sonuç: Konvansiyonel karyotipleme abort mateyalerindeki sitogenetik anomalilerin değerlendirilmesinde halen çok önem taşımaktadır.
Anahtar Kelimeler: otozomal trizomiler, karyotipleme, sitogenetik analiz, spontan abort, poliploidi

ABSTRACT

Objective: Chromosomal abnormalities detected in spontaneous abortion materials were obtained in last ten years period. Details of anomalies, maternal ages and number of pregnancy losses were discussed.
Material and Methods: Cultivation performed from two primer separate flasks and cytogenetic analyses were made via Giemsa banding.
Results: 1046 abortion materials were referred to our Genetic Center for cytogenetic analysis and 1016 cases cultured successfully (97.1%). Chromosomal abnormalities were detected in 212 (20,1%), and number of single autosomal trisomies was 117 (55,2%), among them in 57 cases mothers were older than age of 35. The most common trisomy was 16 (in 22 cases) concordant with the literature. Double and triple aneuploidies were detected in 12 cases (%5,7), monosomy X and polyploidy were found in 27 (12,7%) and 40 cases (18,9%), respectively. Poliploidic aneuploidies were determined in 6 cases with predominantly trisomy 7 plus triploidy. Eight women with previous abnormal karyotypes had an additional abnormality confirmed with their next pregnancies, noted trisomies and poliploidies. Structural abnormalities were detected in 8 cases, and 4 of them were unbalanced. Normal XY karyotypes of the abortion materials were 94, while XX karyotypes were in a huge number, 710, because of the probable contamination of the maternal decidua.
Conclusion: Conventional karyotyping still has high importance to evaluate the cytogenetic abnormalities of the abortion

materials.
Key words: autosomal trisomies, cytogenetic analysis, karyotyping, polyploidy, spontaneous abortion

INTRODUCTİON

Considerable ratio (20-50%) of the spontaneous abortions is due to the chromosomal abnormalities and at least 80% of these aberrations are numerical (1-4). Of these, trisomy is far the most common. The mechanism underlying most cases of trisomy is non-disjunction during meiosis 1 or 2; therefore an augmentation risk of trisomy is expected in relation to advanced maternal age. The underlying cause of non-disjunction is not clear but the lengthy period of maternal meiosis compared with men, is relevant. Monosomy mostly represents the outcome complementary to trisomy from non-disjunction and monosomy X is the single most common chromosomal abnormality among spontaneous abortions, accounting for 13-20% of all abortuses, however autosomal monosomies are infrequently – especially for particular chromosomes as 1 and the reason is unknown (2). Polyploidies are 21% of all numerical abnormalities and regardless from the age of the mother, are mostly due to fertilization errors or post fertilization mitotic cleavage errors (5). Structural abnormalities like deletion, translocation, inversion and duplication detected in pregnancy loss tissues are mostly originated from the parents' chromosomal rearrangements and the remains are de novo. Given the risk of having unbalanced gametes, it is expected an increased frequency of recurrent miscarriages in the carrier couples (5-8).

During the last ten years period, the first or reccurent spontaneous miscarriages were referred to our Genetic Diagnosis Center for cytogenetic analysis. To evaluate the karyotypes of the specimens, the long term tissue cultures were set up and giemsa trypsin bandings were performed. In the present article the details of the cytogenetic results, maternal ages and weeks of gestations of 1016 abortion materials were reported.

MATERİALS and METHODS

Abort materials were obtained after dilatation and curettage and were collected into sterile culture medium and transferred immediately to the laboratory. Informed consents were signed by patients before harvesting the materials and the probabilities of the cell cultures' handicaps were announced. Materials that selected as fetal tissues were cultured in two separate flasks. Colchicine was added to the culture about 3 hours before the harvest procedure and all the steps of the harvest techniques were applied and preparations were completed by Giemsa-Trypsin banding. Cytogenetic analyses were made, at least 20 cells were scored and 5 metaphases were analyzed.

Descriptive statistics and Chi-square test were used for statistical analysis. P value is less than 0.05 was considered statistically significant.

RESULTS

1046 abortion materials were referred to our Genetic Center for cytogenetic analysis in the last ten years period. 1016 of 1046 cases were successfully cultivated (97.1%) and chromosomal abnormalities were detected in 212 (20.1%) (Table1,2). Number of single autosomal trisomies was 117 (55.2%), among them in 57 cases mothers were older than the age of 35 (48.7%) (Table3). The most common trisomy was 16 (in 22 cases) and trisomy 15, trisomy 21 and trisomy 22 were detected in 16, 15 and 13 cases respectively. Double and triple aneuploidies were detected in 12 cases (%5.7). Monosomy X and polyploidy were found in 27 (12.7%) and in 40 cases (18.9%), respectively. Poliploidic

aneuploidies were determined in 6 cases consisting poliploidy plus trisomy of the chromosomes 2, 7, 16 and 20 (Table 1,4).

Table 1 Chromosomal anomalies found in spontaneous abortions

Chromosomal anomalies	**No. Cases**	**%**
Trisomy 16	22	10,4
Trisomy 15	16	7,6
Trisomy 21	15	7,1
Trisomy 22	13	6,1
Other autosomal trisomies	51	24
Total single autosomal trisomies	*117*	*55,2*
Double and Triple aneuploidies	12	5,7
Monosomy X	27	12,7
47,XXY	2	0,9
Poliploidy	40	18,9
Poliploidy +aneuploidy [a]	6	2,8
Total numerical anomalies	*204*	*96,2*
Structural anomalies [b]	8	3,8
Total chromosomal anomalies	*212*	*100*

[a] karyotypes: 70,XXY,+7 (3 cases); 69,XXX,+16,-10; 70,XXY,+20; 70,XXX,+2
[b] including balanced translocations (4 cases), inversions (2 cases), marker (1 case) and deletion (1 case)

Table 2 Cytogenetic abnormalities and number of pregnancy loses

Number of pregnancy losses	**Anormal karyotypes**			**Normal karyotypes**		
	No.cases	**%**	**Mean Gestational age**	**No.cases**	**%**	**Mean Gestational age**
<2	118	55,7	8,8 (5-18)	440	54,7	8,6 (5-18)
≥2	94	44,3	8,3 (5-18)	364	45,3	8,3 (5-15)
Total	212	100		804	100	

No significant difference in number of pregnancy losses between the abnormal and normal karyotyped patients ($\chi^2 = 0,06$, p=0,807)

Table 3 The comparison of autosomal trisomies with normal karyotypes in young age and advanced maternal age women

Maternal age	Autosomal trisomies			Normal karyotype		
	No.cases	%	Mean gestational age	No.cases	%	Mean gestational age
<35	60	51,3	8,2 (5-15)	583	72,5	8,4 (5-18)
≥35	57	48,7	9,2 (6-18)	221	27,5	8,3 (5-18)
Total cases	117	100		804	100	

Relationship between advanced maternal age and increased risk of trisomy is significant.
(p=0,00001, OR=2,51, CI: 1,66-3,79)

Table 4 Recurrent pregnancy losses with recurrent chromosomal anomalies

No. cases	History of pregnancy	Karyotypes of the referred abortions		Maternal age		Gestational age	
		First	Second	First	Second	First	Second
1	G5P2A2L2 D&C=1	92,XXXX	47,XX,+6	35	36	12	11
2	G3P1A2L1	47,XY+20	47,XY,+3	39	40	6	6
3	G2P0A2L0	47,XY,+16	92,XXXX	27	28	10	10
4	G2P0A2L0	46,XX/92,XXXX	47,XX,+22	35	35	10	9
5	G2P0A2L0	47,XY,+21	47,XY,+15	43	44	12	9
6	G2P0A2L0	47,XX,+15/48,XX,+2,+15	47,XX,+16	40	40	8	8
7	G4P1A3L1	45,X/47,XX,+3	69,XXY	33	34	9	10
8	G5P1A3L1 D&C=1	69,XXX,+16,-10	69,XXY	38	39	10	11

G: gravity P: parity A: abortion L: live birth, D&C: dilatation and curettage

Abnormal karyotyped women with young maternal age (<35) and advanced maternal age (≥35) had minimum gestation age of 5 and 6 and maximum gestation age of 15 and 18, respectively (Table 3). Eight women had subsequent pregnancy losses with subsequent abnormal karyotypes, consisting trisomies, triploidies and tetraploidies (Table 1,4). These materials were karyotyped between the 6^{th} and 12^{th} week of gestation, with the mean value of 9.4 week of gestation (Table 4).

Autosomal structural abnormalities were detected in 8 cases; and 4 of them were unbalanced (Table 1). Normal XY karyotypes of the abortion materials were 94, while XX karyotypes were in a huge number, 710.

DİSCUSSİON

Chromosomal abnormalities are the major, 20-57%, cause of spontaneous miscarriages and a large number, 86-94%, of these abortions are due to numerical anomalies (2-4). In our series, approximately every one out of five referred spontaneous abortion materials were found to be cytogenetically abnormal (20.1%). The frequently detected abnormalities are numerical (96.2%), predominantly trisomies with the ratio of fifty-five percent similar with the reported ones (52%) (2,7). More than forty eight percent of our trisomic cases mothers were older than the age of 35 and there was a significant difference in the incidence of trisomies between the advanced maternal age group (35 and up) and the young maternal age group (34 and below)($p<0.05$) (Table 3). The result is concordant with the belief that the risk of trisomy is expected in relation to advanced maternal age (1-4).

Karyotyping the spontaneous abortion materials, first or recurrent ones, has a value for detecting the cytogenetic anomalies (Table 2) (1-4). However it is true that the increasing numbers of previous miscarriages bring an increasing numbers of having abnormal karyotyped pregnancy losses related with the advanced maternal age (9). Concordant with this, six of the eight women who had previous abnormal karyotyped pregnancy losses with an additional abnormality confirmed with their next pregnancies were ≥35 years old (Table 4). One of them (Case 8) had triploidic abortion material accompanying with aneuploidies of chromosomes 10 and 16 (69,XXX,+16,-10) in the first abortion, pure triploidy in the second (Table 1,4). In this study, while pure polyploidies account for 18.9% of chromosomally abnormal spontaneous abortuses, the six couples (consisting case 8) cytogenetic analysis revealed polyplodies plus aneuploidies due to the non-disjunction and fertilization errors in the same pregnancy (Table1). The occurrence of two different mechanisms can confuse the minds and might be explained by the chaotic cytogenetic results of pregnancy loss tissues.

Contamination with maternal deciduas (high about ninety percent) is one of the main problem of long term tissue cell cultures of abortion materials (1, 10-12). To exclude maternal decidual cell contamination (MCC) by using molecular technique, we figured out an accurate algorithm in a research project (10). Need of high experience and efforts of performing molecular techniques and/or their costs, restrict the usage of these methods in many routine laboratories and today MCC is still one of the most important problems in abortion materials cell cultures. The huge number of XX and the small number of XY karyotypes in our study probably due to the problem of MCC. In summary to minimize the contamination of maternal cells in routine analyses, at least the materials must be transported to laboratory immediately in a suitable transport medium and decidual components should be carefully selected for planting.

As a result, conventional karyotyping despite the requirement of tissue cell culture with time consuming, and is prone to maternal contamination, thus over-reporting euploid karyotypes, is still the valuable tool to detect the chromosomal abnormalities of the pregnancy loss tissues.

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