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Prolonged Harvest Time on Amniotic Cell Culture: Is it Offer Important in Prediction of Fetuses with Trisomies?

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OBJECTIVE: In this study, it was objected to investigate the difference in harvest time of amniotic fluids obtained at amniocentesis of normal and trisomic fetuses.

STUDY DESIGN: 113 samples of amniotic fluid were obtained at amniocentesis procedures carried out for several indications at Necmettin Erbakan University Meram Faculty of Medicine, Obstetrics and Gynecology Department between August 2010 to August 2011. Harvest time on amniotic cell culture was both evaluated and compared in 100 samples with normal genetic results and 13 samples with trisomy results.

RESULTS: There were no statistically significant differences between normal and trisomic groups regarding age, gravidity, parity, and gestational week at amniocentesis (p>0.05). Mean harvest time in cultures of amniotic fluids obtained from genetically normal fetuses was 15.84±2.12 days (12-22 days), whereas it was 15.80± 2.47 days (13-20 days) in cultures of amniotic fluids obtained from trisomic gestations. There was no statistically significant difference between groups regarding intervals to harvest.

CONCLUSION: In this study, initial hypothesis was that there may be a possible association of prolonged harvest time with trisomic chromosomal aberrations. Our results concluded that harvest time does not differ between normal and trisomic amniotic fluid cultures.

Key Words: Amniocentesis, Harvest time, Culture, Trisomy

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Introduction

Amniocentesis is an invasive prenatal diagnostic examination widely performed to screen fetal karyotypic abnormalities early in the second trimester of pregnancy. It can be done as early as the 11th to 14th week depending on the medical indication.

Amniotic fluid withdrawn from amniocentesis consists of liquid and cellular components which are separated by centrifugation. After discarding the supernatant, paralel cultures are set up. The growth pattern of amniotic fluid cell lines may vary during culture due to both composition of cell types and environmental conditions. Cultured human primary fibroblasts have limited lifespan in vitro.[1] Fetal cells would be expected to have the longest possible lifespan in vitro, but it is important to recognize that certain genetic and cytogenetic disorders may significantly alter the replication rate and lifespan of cultured cells.[2] The cell-cycle time in trisomic cells of babies with trisomy 18 and 21 was reported to be approximately 3 hours longer than normal controls.[3] Postnatal studies on skin fibroblasts of trisomy 21 patients have shown that significantly decreased number of cell population doublings when compared with age-matched controls.[4]

The aim of the present study was to investigate whether harvest time of amniotic cells obtained from amniocentesis procedures vary between normal and trisomic fetuses.

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Material and Method

Study design and subject selection

113 samples of amniotic fluids withdrawn during amniocentesis procedures were included into the study. The study group consisted of 13 samples from trisomic gestations and 100 samples from gestations with normal karyotype. Amniocenteses were performed between 16 and 20 weeks of gestation at Necmettin Erbakan University, Meram Medical Faculty Hospital, Obstetrics Department because of advanced

maternal age, abnormal ultrasound findings, abnormal maternal serum screening results, a previous child with a congenital anomaly, a family history of chromosome aberrations, or for other reasons. All patients were informed about the procedure and written informed consent was obtained from each patient.

Genetic analysis

Twenty milliliter of amniotic fluid was collected (the first 1-2 ml of amniotic fluid was discarded) and centrifuged at 1000 r/min for 10 minutes. After discarding the supernatant, three cultures were set up. The cultures were grown in three separate culture media in 5 per cent CO2 at 37°C: Chang's medium (Irvine Scientific) for the first flask, Amniomed (Biochrom KG) for the second flask, and Bio-Amp-2 (Biological Industries) for the third. The amniotic fluid cells were allowed to settle for 6-7 days and then fed with fresh medium after 6-7 days before subculturing and harvesting. Chromosomes were harvested according to standard techniques and were analyzed using GTG, CBG, and Ag-NOR banding. Twenty G-banded metaphases from two independent cultures of each sample were analyzed. Chromosome abnormalities detected by karyotype analysis were classified into the following categories: classical autosomal aneuploidies (trisomies 21, 18 and 13), sex chromosome aneuploidies, chromosome rearrangements (translocations, inversions, deletions and duplications), supernumerary marker chromosomes, triploidies, and other chromosome abnormalities (including ring chromosomes, multiple chromosome abnormalities and isochromosomes). Polymorphic variants were not considered as chromosome abnormalities. In this study, harvest time was evaluated only in trisomies and compared with that of normal karyotypes.

Pregnant women with systemic diseases were excluded from the study.

The study was approved by a local ethics committee in Turkey (Meram Medical Faculty's Ethics Committee Ref 2012/22).

Statistical analysis

Values were expressed as mean±SD for continuous variables and number. Harvest time was compared between the study and control group using Student's t-test. All P values were 2-tailed and $P < 0.05$ was considered statistically significant.

Results

The study group consisted of 113 patients and their amniotic fluid samples, 13 samples from trisomies and 100 control samples with normal karyotype. Mean age of the patients with trisomic fetuses was 33.84±6.59 (19-43) and was 32.79±7.42 (18-49). There was no statistically significant difference between groups regarding age, gravidity, parity, gestational week at amniocentesis (summarized at table-1). Indications for amniocenteses are also summarized at table-2.

Table 1: Demographic and clinical characteristics of the patients

	Trisomy group (n=13)	Normal karyotype group (n=100)	P value
Mean age (years)	33.84 ± 6.59 (19-43)	32.79 ± 7.42 (18-49)	0.627
Mean gravidity	2.8 ± 1.2 (1-5)	3.17 ± 1.75 (1-11)	0.523
Mean parity	1.4 ± 1.0 (0-3)	1.6 ± 1.3 (0-8)	0.710
No of Previous miscarriage	0.3 ± 0.7 (0-2)	0.5 ± 0.9 (0-4)	0.510
Live births	1.5 ± 1.1 (0-4)	1.5 ± 1.3 (0-8)	0.941
Gestational week at amniocentesis	17.0±0.8 (16-18)	17.2 ± 1.2 (16-20)	0.584

Table 2: Indications for amniocenteses

Indications	Trisomy group (n=13) (N/%)		Normal karyotype group (n=100) (N/%)	
Triple test positivity	4	(30.8%)	34	(34%)
Increased maternal age	6	(46.2%)	49	(49%)
Double test positivity	0	(0%)	6	(6%)
Previous Down History	0	(0%)	3	(3%)
Anomaly at USG	1	(7.6%)	5	(5%)
Increased nuchal translucency	2	(15.4%)	3	(3%)
Total	**13**	**(100%)**	**100**	**(100%)**

Mean harvest time in amniotic fluid cultures of trisomic fetuses was 15.84±2.47 days (13-20), whereas it was 15.8±1.12 days (12-22) in cultures of fetuses with normal karyotypes. There was no statistically significant difference between groups regarding harvest time on amniotic fluid cultures (p=0,942).

Discussion

Initial hypothesis of the study was conducted as ''chromosomal aberrations may influence and delay harvest time on cultures of amniotic fluids''. So harvest time of amniotic cell cultures from trisomic gestations was analyzed and compared with control in this report. There is no other report in literature about the ongoing of cell cultures obtained from chromosomally abnormal amniotic fluid.

There are limited reports about the behaviour of cell cultures after amniocentesis and the information is limited about normal karyotypes. In these reports subculture time and harvest time were evaluated in normal karyotype by comparing different culture media.[5-7]

Regarding association of cell culture variability and congenital anomalies, there are few reports about the detection of extra metacentric microchromosome in cell culture which was found to be associated with chromosomal aberrations.[8]

The present study is the first to analyze the behaviour of cell cultures and harvest time of trisomic gestations after amniocentesis performed in second trimester and shows no statistically significant difference when compared with the group that had normal karyotype.

In conclusion, delay in subculture and harvest time does not predict chromosomal aberrations.

Amnion Hücre Kültüründe Uzamış Harvest Zamanı Trisomik Fetusların Öngörülmesinde Yardımcı Olabilir mi?

AMAÇ: Bu çalışmada, normal ve trisomik fetuslardan alınan amniotic sıvılarda harvest zamanları arasındaki farklılığı araştırmayı amaçladık.

GEREÇ VE YÖNTEM: Necmettin Erbakan Üniversitesi Meram Tıp Fakültesi Kadın Hastalıkları ve Doğum kliniğinde Ağustos 2010- Ağustos 2011 tarihleri çeşitli endikasyonlar ile gerçekleştirilen amniosentez işlemlerinden toplam 113 amnion sıvı örneği alındı. 13 trisomik fetusa ve 100 normal fetusa ait amnion hücre kültürlerinde harvest zamanları karşılaştırıldı.

BULGULAR: Trisomik hasta grubu ve normal kontrol grubu arasında yaş, gravidite, parite ve amniosentez esnasındaki gestasyonel yaş açısından istatistiki olarak anlamlı farklılık tespit edilmedi (p>0,05). Genetik olarak normal olan fetuslardan alınan amnion hücre kültürlerinde ortalama harvest zamanı 15.84±2.12 gün (12-22 gün), trisomik fetuslara ait amnion hücre kültürlerinde ise 15.80±2.47 gün (13-20 gün) idi. Her iki grup arasında harvest zamanları açısından istatistiki olarak anlamlı farklılık tespit edilmedi.

SONUÇ: Bu çalışmada, başlangıç hipotezi trisomik fetuslara ait amnion hücre kültürlerinde harvest zamanının uzayabileceği yönünde idi. Sonuçlarımız normal fetuslara ait hücre kültürleri ile trisomik fetuslara ait amnion hücre kültürlerinde harvest zamanının farklılık göstermediğini desteklemektedir.

Anahtar Kelimeler: Amniosentez, Harvest zamanı, Trizomi

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