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Investigation of the diagnostic value of chromosome analysis and bacterial artificial chromosome-based array comparative genomic hybridization in prenatal diagnosis

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Background/aim: To investigate the diagnostic value of bacterial artificial chromosome (BAC)-based array comparative genomic hybridization (CGH) and chromosome analysis in prenatal diagnosis.

Materials and methods: This study included the chromosome analysis and BAC-based array CGH analysis of 140 amniocentesis samples with prenatal diagnosis indications.

Results: Karyotype analysis showed trisomy 21 in 4 patients, trisomy 18 in 5 patients, monosomy X in 1 patient, and other anomalies in 3 patients. The BAC-based array CGH analysis showed 4 patients with trisomy 21, 4 patients with trisomy 18, and 1 patient with monosomy X as a numerical chromosome anomaly, while partial duplication was observed in chromosome 14 in 1 case as a structural anomaly.

Conclusion: The array CGH is the most effective method available to complement cases where chromosome analysis, a gold standard in prenatal diagnosis, proves to be insufficient. Considering the inherent limitations of both methods, complementary features should be introduced in order to be able to give the most accurate data at the right time.

Key words: Prenatal diagnosis, bacterial artificial chromosome-based array comparative genomic hybridization, karyotyping

1. Introduction

The main target of prenatal diagnosis is to diagnose anomalies of the fetus in the early period and to enable the parents to make their own decisions regarding the future of the fetus within the framework of personal, social, and ethical principles (1).

Prenatal diagnostic methods are classified as noninvasive and invasive techniques. Ultrasonography and biochemical tests performed on the mother's blood are the principal prenatal procedures. Invasive methods such as amniocentesis or cordocentesis are used to gather more information about fetal karyotype (2).

Fetal karyotyping is offered to pregnant women with an elevated risk of carrying fetuses with chromosomal anomalies due to advanced maternal age, abnormal maternal serum screening results, abnormal ultrasonography findings, or family history of chromosome anomaly (3).

Chromosome analysis is the most common method in prenatal diagnosis for genetic testing. This method can detect all chromosome aneuploidies and structural changes larger than approximately 5 Mb. Chromosome analysis is a procedure requiring culturing, with a longer analysis process compared with molecular procedures. Although all common aneuploidies can be detected with this method, the long reporting process required the development of diagnosis methods that could give quicker results. The main procedures developed to address this problem include fluorescent in situ hybridization (FISH), quantitative polymerase chain reaction (QF-PCR), and array-comparative genomic hybridization (aCGH) methods. The use of the aCGH method, which allows for detection at the whole-genome level, has become more common in prenatal diagnosis (4–6).

The aCGH technique is a molecular cytogenetic method originating from FISH, showing fluorescent color differences acquired by binding test (patient) and reference DNA samples stained with different fluorescent dyes (6).

The aCGH method is performed using the platforms where relevant DNA series are spotted. Genomic clones with large DNA fragments (bacterial artificial chromosomes/P1 artificial chromosomes, BACs/PACs) or DNA microarrays with smaller PCR products are generated as targets for hybridization (7).

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It is possible to use aCGH, also called molecular karyotyping, to screen genome-wide segmental genomic copy number variations, such as deletions and duplications, and also all aneuploidies (8,9).

This study aims to investigate the efficiency of chromosome analysis in the diagnosis of genetic disorders observed in the prenatal period and BAC-based aCGH techniques, which have become more popular in recent years, in prenatal diagnosis.

2. Materials and methods

2.1. Materials

In the Medical Genetics Laboratory of Kocaeli University we performed both chromosome analysis and BAC aCGH for 140 patients who applied to our clinic in between 2011 and 2012. All patients underwent pretest counseling. This study was approved by the Local Research Ethics Committee (KOU KAEK 2012/157).

2.2. Methods

2.2.1. Chromosome analysis

Chromosome analysis was performed according to standard methods using cultured cells from 10 mL of amniotic fluid. G-banded chromosomes were analyzed and recorded according to the International System for Human Cytogenetic Nomenclature (ISCN) 2008.

2.2.2. BAC-based aCGH

For aCGH, 5 mL of amniotic fluid was used. Amniocentesis was done only once for each subject.

Genomic DNA was obtained using the Magna Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics GmbH, Germany). Wave length absorbances of 260 and 280 nm were measured by spectrophotometer (NanoDrop, ND-1000). gDNAs with an A260/A280 ratio between 1.7 and 2.0 were used. References and samples were labeled with Cy5-dCTP and Cy3-dCTP using the Fluorescent Labelling System (dCTP/BAC) Kit (BlueGnome Ltd., Cambridge, UK). After incubating for 16 to 20 h at 37 °C, samples were purified with AutoSeq G50 columns (BlueGnome Ltd.). Human Cot-I DNA (BlueGnome Ltd.) was added to avoid consecutive matches from repetitive regions. Hybridization mixture was added and denatured at 75 °C. Samples were loaded on Cytochip Focus Constitutional V1.11 platforms (BlueGnome Ltd.) and hybridized at 47 °C for 16–21 h. Finally, the platforms were scanned (Agilent Microarray Scanner; Agilent Technologies, Palo Alto, CA, USA) and analyzed by BlueFuse Multi v2.1 software (BlueGnome Ltd.).

3. Results

The prenatal diagnosis indications of the study group included advanced maternal age, high double-triple screening test results, abnormal ultrasound findings, family history of an anomaly, family history of mental retardation, and a family history of chromosome anomaly. The distribution of cases by indications in the study group is listed in Table 1.

Although the gestational age in cases of amniocentesis varied between 12 and 28 weeks, the mean age was calculated to be 20 weeks. The ages of mothers varied between 18 and 46 years old, with the mean maternal age being 32. Cell culture process performance was found to be 97% (136/140) and the prenatal diagnostic rate was 99% (139/140) in the amniocentesis series. A specific FISH method was conducted on chromosomes 13 and 21 as well as BAC-based aCGH in 3 of the cases where no metaphases could be obtained for evaluation from cell cultures. However, no analysis could be performed by any method in 2 of the submitted samples, which were contaminated with blood.

Chromosomal anomalies were detected in 14 (9%) of 140 cases through results from applied cytogenetic studies for prenatal diagnosis. Three of these chromosome anomalies were structural anomalies, while 10 (76%) of them were numerical ones. The most common anomalies were karyotypes with trisomy 21 and trisomy 18 numerical anomalies, while structural anomalies were observed at an equal rate (7%).

Trisomy 21 was detected in 4 cases, trisomy 18 in 4 cases, and monosomy X in 1 case as a result of the BAC array CGH analysis. BAC array and chromosome analysis results are listed in Table 2.

4. Discussion

At least one prenatal diagnosis indication was found in each of the cases in this study. An abnormal screening test result (69%) was the most frequently observed indication, with abnormal ultrasound findings (21%) ranking second. Advanced maternal age ranked third at 11%. Both incidence rate and incidence order differ among genetic diagnosis centers throughout Turkey and in other

Table 1. Distribution of cases by indication.

Indication	%	n
Advanced maternal age	11.9	17
Abnormal serum screen	69	98
Abnormal ultrasound findings	21	30
Habitual abortion	0.7	1
Previous child with anomaly	1.4	2
Family history of mental retardation	0.7	1
Previous child with chromosome anomaly	0.7	1

Table 2. Karyotype and BAC aCGH results of abnormal cases.

Indicator	Karyotype	BAC array CGH result
Abnormal serum screen	47,XX,+21[20]	Duplication of whole chr. 21
Advanced maternal age, abnormal ultrasound findings	47,XY,+18[30]	Duplication of whole chr. 18
Abnormal serum screen	Mosaic karyotype, 47,XY,+18[2]/46,XY[53]	Normal
Advanced maternal age	47,XX,rob(21;21)(q10;q10)[20]	Duplication of whole chr. 21
Abnormal serum screen	47,XY,+18[20]	Duplication of whole chr. 18
Abnormal serum screen	47,XY,+18[20]	Duplication of whole chr. 18
Abnormal serum screen	47,XY,+21[20]	Duplication of whole chr. 21
Abnormal serum screen	47,XX,+2[9]/46,XX[30]	Normal
Abnormal serum screen	46,XY,del(1)(q23)[2]/46,XY[98]	Normal
Abnormal serum screen	47,XY,+18[85]/46,XY[3]	Duplication of whole chr. 18
Abnormal serum screen, Abnormal ultrasound findings	47,XX,+18[20]	Duplication of whole chr. 18
Abnormal serum screen	46,XY,inv(4)(p13q21)[20]	Normal
Abnormal serum screen	47,XX,+21[20]	Duplication of whole chr. 21
Abnormal ultrasound findings	45,X[20]	Deletion of whole chr. X
Family history of mental retardation	47,XY,+mar[20]	Duplication of 14q11

countries. A study conducted in Turkey found, in contrast to the findings of this study, high screening test results to be 54.9%, advanced maternal age to be 20%, and abnormal USG findings to be 16% (10). Tongsong et al., in a 1998 study, differed dramatically in their findings and reported advanced maternal age to be 86% (11). It is striking that in our study the acceptance rate of advanced maternal age as an indication was reduced and that it was screening tests and ultrasound findings that led to the prenatal diagnosis. In their studies covering the subject matter, Dommergues et al. concluded that amniocentesis should not be a routine procedure in women with advanced maternal age, but that it should be selectively recommended based on the results of noninvasive screening tests (12).

Chromosome anomaly was seen in 3 of 17 cases (17%) with amniocentesis both in karyotyping and in BAC-based aCGH analysis due to advanced maternal age. In a study conducted with 356 cases, Yüce et al. concluded that 1.2% of 158 cases with amniocentesis and chromosome analysis due to advanced maternal age had a chromosome anomaly (13). Api et al. found this rate to be 2.7% (14). We consider that the high rate found in this study compared with similar studies was due to the presence of additional indications, such as high screening test results and fetal anomalies.

Ultrasonography is an important constituent of the noninvasive technique. Frequently observed ultrasound findings in fetal chromosome findings can be listed as increase in nuchal translucency, nonappearance of nasal bone, choroid plexus cyst, and cystic hygroma. Chromosome anomaly is only seen in 5% of cases with abnormal ultrasound findings when considering all ultrasound findings.

Amniocentesis was applied in 98 cases due to the high screening test results and numerical and/or structural chromosome anomaly was detected in 9 (10%) cases. Yüce et al. recorded this rate as 3% in another study (13).

Cytogenetic analysis is primarily conducted in high-risk pregnancies. Nevertheless, a majority of patients undergoing applied cytogenetic analysis have a normal karyotype, but chromosomal alterations are observed on a microscopic level in 5%–10% of cases (15). In this study, this rate was found to be 9%, in conformity with the findings of other studies.

Chromosome anomalies are responsible for different complex phenotypes, such as mental retardation and birth defects. While 80% of chromosome anomalies seen in neonates are composed of trisomy 21, trisomy 18, and trisomy 13 as autosomal aneuploidies, sex chromosome anomalies such as Turner syndrome and Klinefelter syndrome make up the remaining ones.

Down syndrome is the most commonly seen chromosome anomaly in prenatal diagnosis. Trisomy 21 was observed in 4 cases in our study group. All of these findings were specified with chromosome analysis and the BAC array. Three of them were counted as the regular type and one was a de novo Robertsonian translocation.

Cells were required to be grown in a cell culture in order to raise the number of living cells in the amniotic liquid sample for chromosome analysis. This procedure lasted approximately 10–15 days. The anxiety levels of the expectant mothers undergoing applied amniocentesis

increased during this period. Moreover, time is of great importance in the later gestational weeks, because termination becomes harder in the case of a possible anomaly if amniocentesis is applied in this period. Therefore, techniques to get results more rapidly have been developed. Quicker methods, requiring less endeavor, are needed for microscopic karyotype analysis in order to obtain results within the targeted period in prenatal tests.

The aCGH technology is available for use in prenatal diagnosis in order to research structural chromosome anomalies associated with genome-wide copy number changes. The principle behind the aCGH technology depends on the comparison of patients' genomic DNAs that have chromosomal deletion and duplications with the same amounts from healthy samples. Imbalanced chromosomal anomalies causing simultaneously aneuploidies of all chromosomes and alterations in the copy number can be detected with the aCGH method in a short time (16,17).

Amnion cell culturing both takes time and does not always present successful results. The risk of failure in culturing, encountered in all laboratories, increases the anxiety of families, because another invasive procedure may be needed in such a situation and the risks associated with this intervention are repeated.

In our study, the culture success rate was 96.4% and the success in getting final diagnostic results was 98.5%. Similarly, Saatçi et al. reported the cell culture success rate as 97% (18). FISH and BAC-based aCGH were applied in cases that displayed no metaphase, and thus a difference between these rates occurred and patients were informed accordingly. Materials that were contaminated with maternal blood had no procedures applied to them, due to the nonconformity with molecular tests.

Mosaicism may be the main problem in the prenatal diagnosis due to the hard-to-predict phenotypic effect of karyotypes. There were 3 distinct cases with mosaic karyotype in the study group. Two of them were numerical and one of them was a structural anomaly. These mosaic karyotypes could not be detected by aCGH. Detection of mosaicism under 10% is a limitation of aCGH technology.

Balanced chromosome anomalies such as translocations and inversions cannot be detected by aCGH. In prenatal diagnosis, when balanced chromosome anomalies are detected by chromosome analysis, parental chromosome analysis is also necessary. If parents have normal karyotypes, reporting can be more complex, because it is reported that 6.1% of fetuses with translocations such as de novo balanced chromosome anomaly carriers will have abnormal phenotypes. In a study by Lee et al., de novo balanced translocations were seen in 17 fetuses and 2 of them had submicroscopic deletions at translocation breakpoints. These deletions were obtained only by aCGH. Balanced chromosome anomaly was detected in only one case in our group. Inv (4) (p13q21) was observed in a patient admitted with a high risk of trisomy 21. Inverted chromosomes could not be identified by aCGH, as expected. On the other hand, no submicroscopic deletions or duplications were found in this case. The aCGH method can be useful in cases of de novo balanced chromosome anomaly (19,20).

Marker chromosomes are composed of chromosomes of unknown origin. The phenotypic effect becomes unpredictable if the origin of the increasing genetic material is unknown, and aCGH may help in identification of marker chromosomes depending on the euchromatin participation and array resolution. Chromosome analysis revealed a marker chromosome in one case when applied to amniocentesis material. The origin of extra chromosome parts was identified as belonging to the 14q11 area by BAC aCGH. As a result of parental chromosome analysis, t(7;14) was found in expectant mothers, and the part of the chromosome appearing as a marker chromosome was understood to be a derivative of chromosome 14.

The BAC aCGH displays genome-wide copy number changes. The probability of encountering copy number changes in such benign groups at, for example, an oligo-based array platform, particularly with high resolution, is higher than with a BAC-based array platform. Alterations in copy number are classified as a pathological group with known or unknown clinical effects and a benign group with no or unknown clinical effects. Copy numbers both in pathological and benign groups can be detected by this method (16,17). No alteration of copy number with suspicious phenotypic effect was observed in the study group. It has been recorded that the detection of small-scale changes that is impossible by chromosome analysis, and of changes with unknown prognosis and of changes associated with no definite syndrome, results in possible challenges in genetic counseling, especially in the prenatal diagnosis period (21,22).

Array CGH has the advantage of being able to show results in 3 days but it has limitations in detecting low-rate mosaicisms and balanced rearrangements such as translocations and inversions. Our center has experience with over 2500 aCGH samples to help to overcome difficulties in bioinformatics analysis. However, it is still a serious problem for us to interpret copy-number variation data and unknown aberrations. BAC arrays have lower resolutions than oligo-arrays but are capable of detecting big rearrangements of over 100,000 base pairs, since they have been produced by FISH probe sources. Thus, BAC arrays are much more reliable in making comments on diagnostic purposed studies.

Array CGH has a higher diagnostic capacity than chromosome analysis for detecting chromosomal

alterations in fetuses with normal karyotypes and abnormal ultrasound findings. On the other hand, aCGH is unable to screen balanced chromosomal alterations such as triploidy and low-rate mosaicism, which can be detected by chromosome analysis. However, both methods have several limitations, and we think that using these methods together gives the right information in routine prenatal diagnosis. Unfortunately, aCGH platforms are still 10 times more expensive than cytogenetic studies. However, the molecular diagnostic industry offers new options in the next generation of sequencing, which will be available at reasonable prices in the coming decades.

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