Chromosome Analysis of Uncultured Amniocytes by Comparative Genomic Hybridization in Early Amniocentesis

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Objective: *To study chromosome analysis by comparative genomic hybridization (CGH) compared with the conventional technique in early amniocentesis.*

Material and Method: *Cross-sectional descriptive study design was performed in 32 singleton pregnant women with gestational age between 12-15 weeks. Transabdominal amniocentesis was carried out under ultrasound guidance. The amniotic fluid samples were simultaneously investigated using CGH and the conventional cytogenetics study as a gold standard.*

Results: *Amniocentesis were done for advanced maternal age in all cases. The mean maternal age was 35.8 years (35-42 years). The mean gestational age was 13.7 weeks (12-15 weeks) .The chromosome analysis by CGH technique of uncultured amniocyte showed 17 normal female chromosomes (53.1%) and 15 normal male chromosomes (46.9%). This finding was the same as the conventional cytogenetics method. The mean duration of the CGH method was 6 days and that of the conventional cytogenetics method was 13.7 days (10-23 days).*

Conclusion: *The CGH technique is a reliable technique for a rapid prenatal diagnosis of chromosome study in early gestation.*

Keywords: *Chromosome, Early Amniocentesis, Comparative Genomic Hybridization*

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Amniocentesis is the most commonly performed invasive test for prenatal diagnosis of genetic disease, especially for chromosome abnormality. The standard amniocentesis is performed in the mid trimester between 16-18 weeks of gestation and the period from sampling to get the result is about 2-3 weeks. By that time the pregnancy is already evident, the mother feels the baby movement, and considerable “attachment” to the fetus already exists. The implications of an abnormal result as this gestation are emotional damage to the parents and also increased maternal

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risk from pregnancy interruption at mid trimester. Early prenatal diagnosis offers the advantage of a safer termination of pregnancy with reduced social and psychological trauma. The first trimester prenatal diagnosis has been investigated including the chorionic villus sampling (CVS) and early amniocentesis. However, the CVS is complicated by higher procedure related fetal loss rate, more frequent maternal cell contamination, and placental mosaicism[1], and also increased risk of severe limb defects in the neonate[2]. As an alternative to CVS, the early amniocentesis at 11-14 weeks of gestation will be the procedure of choice for prenatal diagnosis[3]. But limiting the volume of the amniotic fluid specimen can cause low cell count and consequently cause culture failure and long duration of culture time for chromosome analysis[4,5]. Comparative genomic hybridization (CGH) is a new molecular cytogenetic technique which can detect and map whole and partial aneuploidies throughout a genomic specimen DNA without culturing the specimen cells[6,7]. This technique is unlike standard cytogenetics because actively dividing cells are not necessary. Chromosomal copy number changes of DNA sequences can be detected without having to selectively perform fluorescence in situ hybridization (FISH) for a specific chromosomal sequence[8,9]. However, CGH cannot be used to detect balanced translocations, inversions or identification of specific chromosome rearrangements. CGH can be used as a comprehensive test for detection of prenatal unbalanced chromosomal abnormalities detection within 3-4 days and using 4-5 ml of the specimen[10]. CGH should be another useful rapid test for the detection of unbalanced chromosomal abnormality in early gestation. The goal of this present study is a study of chromosome analysis by comparative genomic hybridization (CGH) compared with conventional technique in early amniocentesis for both the accuracy and duration of the test.

Material and Method

A prospective descriptive study was carried out at the antenatal clinic, Phramongkutklao Hospital between June 1st and November 30th 2002. The protocol was approved by the Royal Thai Army Medical Department Institutional review boards.

The authors included 32 pregnant women at 12-15 weeks of gestation who were referred for prenatal diagnosis of chromosome abnormality. The indication for referring were advanced maternal age (35 years old and older at the expected date of delivery). The indication criteria to enter the study were singleton pregnancy without medical nor obstetrical complication. Patient and husband decided to have the early prenatal diagnosis test after genetic counseling and the informed consent was obtained. Transabdominal amniocentesis was carried out by the sterile technique under ultrasound guidance using a 20 gauge disposable sterile spinal needle. Approximately 0.5-1 ml of amniotic fluid was initially drawn and discarded to prevent maternal cell contamination and 12-15 ml of amniotic fluid were obtained. The amniotic fluid samples were simultaneously processed for CGH and conventional cytogenetics by two independent investigators. Both investigators performed the test in a separate laboratory and were also blinded for the result of the other test.

CGH protocol

The specimen increased the amount of DNA by polymerase chain reaction (PCR) technique. The principle of CGH involves two color fluorescence in situ hybridization of specimens and reference DNA to a normal male metaphase chromosomes. To prevent non-specific hybridization, the differentially labeled specimen and control DNA are mixed together with Cot-1 DNA (containing repetitive sequences of the genome). Images of metaphases spreads are obtained with a cooled charge-couple device (CCD) camera and

fluorochrome-specific optical filters to capture FITC (fluorescein isothiocyanate) and TRITC (tetramethylrhodamine isothiocyanate) fluorescence. The copy number changes in the genome relative to the normal are assessed based on the differences in fluorescence intensities along the chromosome (Fig. 1.). The fluorescence ratio profiles for each chromosome were calculated using the program CGH by metasystem. Metaphases spreads with uniform high intensity fluorescence in both green and red colors on both homologous chromosomes and with no background spots, were selected for evaluation. Details of the CGH method were described[11].

incubator for 7 days, the cell growth was then evaluated under an inverted microscope and the medium were changed every 2 days. The harvesting was performed when at least 10 medium colonies containing mitotic cells were observed by the experienced cytogenetic technician. The karyotyping was undertaking by Trypsin and Giemsa banding technique.

Results

A total of 32 patients were enrolled in the present study. The maternal age 35 years old or more was the indication for prenatal diagnosis. The mean maternal age was 35.8 years (35-42

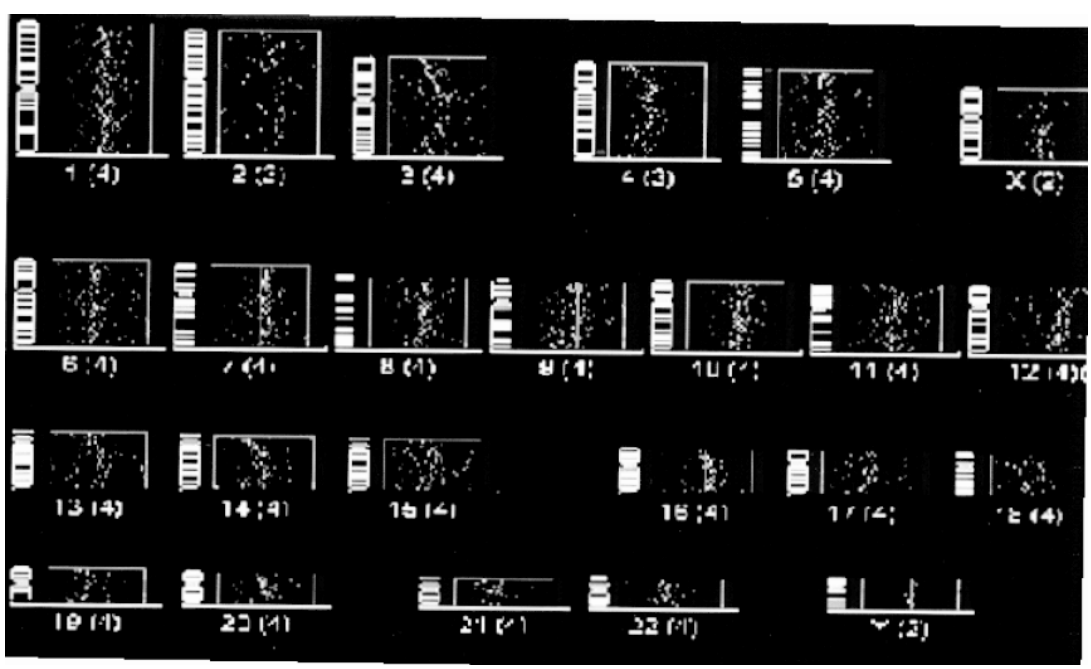

Fig. 1 The CGH analysis measure the intensity of fluorochroms along each chromosome using the computer ISIS software (metasystems)

Cytogenetics conventional protocol

The amniotic fluid specimen was centrifuged at 1,100 rpm for 10 minutes, then the cell pellet was cultured into the flasket using the Amniomax for a culture medium. The cultures were incubated at 37 degrees celcius in the CO_2

years). The mean gestation age was 13.7 weeks (12-15 weeks). All cases were successfully analyzed by using conventional cytogenetics and CGH. The conventional cytogenetics methods were completed in 10-23 days, and the mean duration was 13.7 days. Meanwhile, the result of CGH was obtained

Table 1. Comparison of conventional karyotyping and CGH results

Conventional result	Conventional karyotype	CGH
46, XY	15	15
46, XX	17	17

CGH = comparative genomic hybridization

within 6 days. The conventional karyotype results compared with those obtained by CGH analysis, the results are summarized in Table 1. The CGH provided the same result as the conventional method in all cases.

All were scanned by ultrasound examination at 20 weeks of gestation, including fetal biometry, amniotic fluid volume and fetal anomaly scan. All were found to have normal ultrasonographic examination. None had complications related to the procedure especially the leaking of amniotic fluid in this present study.

Discussion

The authors reported the successful prenatal diagnosis of chromosome study by CGH method in the gestational age 12-15 weeks. The present study was performed on early gestational age and small amounts of amniotic fluid were obtained (1 ml per week of gestation) in order to minimize the fetal complication[12,13]. Eight to ten ml of amniotic fluid was sent for the conventional cytogenetics study. Only 4-5 ml was available to study the CGH. The PCR technique was performed in order to increase the amount of DNA in the specimen. This made the duration of the present CGH study longer than the previous report by Lapierre[10]. The results of the conventional cytogenetics were obtained in all cases with the same result as the CGH. However, the mean duration of the conventional karyotyping was 13.7 days (10-23 days). The CGH was confirmed to be rapid test for chromosome analysis in the early amniocentesis. The chromosome results in the present study were normal in either male karyotype (46XY) or female karyotype (46 XX). The present study was not able to demonstrate the accuracy of the CGH for abnormal chromosome such as trisomy, monosomy, translocation and inversion. Further study needs to be performed on a large sample size in order to study the sensitivity and specificity of the CGH.

In the present study, the CGH was a reliable and rapid test for prenatal diagnosis of chromosome study in an early gestation.

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การตรวจวิเคราะห์โครโมโซมจากเซลล์น้ำคร่ำที่ไม่ได้เพาะเลี้ยงซึ่งได้จากการเจาะน้ำคร่ำในระยะแรกของการตั้งครรภ์ไตรมาสที่สอง โดยวิธีการตรวจแบบ Comparative Genomic Hybridization

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วัตถุประสงค์: *เพื่อศึกษาการตรวจวิเคราะห์โครโมโซมจากเซลล์น้ำคร่ำ โดยวิธีการตรวจแบบ comparative genomic hybridization (CGH) เปรียบเทียบกับวิธีมาตรฐาน ในระยะแรกของการตั้งครรภ์ไตรมาสที่สอง*

วัสดุและวิธีการ: *ใช้รูปแบบการศึกษา cross-sectional descriptive โดยศึกษาในสตรีตั้งครรภ์เดี่ยว 32 ราย ซึ่งได้รับการเจาะตรวจน้ำคร่ำผ่านทางหน้าท้อง เมื่ออายุครรภ์ระหว่าง 12-15 สัปดาห์ โดยใช้การตรวจคลื่นเสียงความถี่สูง และนำน้ำคร่ำที่ได้ไปตรวจด้วยวิธี CGH และการตรวจด้วยวิธีมาตรฐานทางเซลล์พันธุศาสตร์ เป็นวิธีการตรวจมาตรฐาน*

ผลการศึกษา: *ข้อบ่งชี้ในการเจาะน้ำคร่ำในผู้ป่วยทุกราย คือ มารดาอายุมาก อายุเฉลี่ย 35.8 ปี (35 - 42 ปี) อายุครรภ์เฉลี่ย 13.7 สัปดาห์ (12 - 15 สัปดาห์) การตรวจวิเคราะห์โครโมโซมในเซลล์น้ำคร่ำที่ไม่ได้เพาะเลี้ยงพบโครโมโซมปกติซึ่งเป็นเพศหญิง 17 ราย (ร้อยละ 53.1) และโครโมโซมปกติซึ่งเป็นเพศชาย 15 ราย (ร้อยละ 46.9) ซึ่งให้ผลเช่นเดียวกับการตรวจด้วยวิธีมาตรฐาน ในการตรวจด้วยวิธี CGH ใช้ระยะเวลาโดยเฉลี่ย 6 วัน ส่วนการตรวจด้วยวิธีมาตรฐานใช้ระยะเวลาเฉลี่ย 13.7 วัน (10 - 23 วัน)*

สรุป: *การตรวจวิเคราะห์โครโมโซมในเซลล์น้ำคร่ำระยะแรกของการตั้งครรภ์โดยวิธีการตรวจแบบ CGH เป็นวิธีที่ได้ผลรวดเร็วและเชื่อถือได้*