Chorionic Villus Sampling for Early Prenatal Diagnosis at Bhumibol Adulyadej Hospital

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Objective: *To evaluate results of chorionic villus sampling for early prenatal diagnosis at Bhumibol Adulyadej Hospital.*
Design: *Retrospective descriptive study.*
Setting: *Perinatal unit, Department of Obstetrics and Gynaecology, Bhumibol Adulyadej Hospital.*
Subjects: *Three hundred and eighty three women were enrolled to chorionic villus sampling at the perinatal unit, Department of Obstetrics and Gynecology, Bhumibol Adulyadej Hospital, from November 10,1997 to October 17, 2006.*
Results: *During the present study periods three hundred and eighty three women were recruited, of these chorionic villus sampling for chromosome diagnosis were performed on 355 while 6 were for abnormal Thalassemia screening. Twenty two cases were excluded because ultrasound examination showed anembryonic pregnancy or fetal demise in utero in 13 cases, multiple fibroids in 4 cases, large area of placental hemorrhage in 3 cases, 1 case of multiple pregnancy and in 1 case the placenta was in an inappropriate position. The most common indication was elderly gravidarum (95.84%). Other indications were abnormal Thalassemia screening, abnormal ultrasound findings, family chromosome disorder, previous Down syndrome, and severe oligohydramnios. The authors found eleven cases of chromosome abnormalities, four cases of maternal cell contamination and three cases of failed tissue culture (two cases from transcervical chorionic villus sampling and one case from transabdominal chorionic villus sampling) and two cases of mosaicism. There were two fetal losses in the present study and all the babies from the normal chromosome result looked normal. Second trimester amniocentesis following chorionic villus sampling was required due to maternal cell contamination, mosaicism and failed tissue culture.(2.77%) All cases had follow-up ultrasound scan during 18-20 weeks.*
Conclusion: *The authors found that chorionic villus sampling is a possible alternative technique for prenatal diagnosis of cytogenetic abnormalities and abnormal Thalassemia screening in Thailand. It probably has a slightly higher rate of failed tissue culture and maternal cell contamination than amniocentesis, but it is generally done earlier in pregnancy than amniocentesis and is particularly advantageous for detecting certain genetic conditions.*

Keywords: *Prenatal diagnosis, Chorionic villus sampling*

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Chorionic villus sampling (CVS) and amniocentesis are prenatal diagnostic procedures used to detect certain fetal genetic abnormalities. Both procedures increase the risk for miscarriage[1,2]. The ability to diagnose fetal disorders by karyotyping, molecular and biochemical analysis is one of the most rapidly growing clinical applications for genetic diseases. Over the last decade, techniques of first trimester fetal tissue sampling have enabled diagnosis of many genetic disorders to be made earlier in pregnancy thus allowing patients to have the option of a safe and less psychological trauma from pregnancy termination if the fetus is affected[3,4]. More than 80,000 procedures were

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reported to the International Registry from 1983-1992[5]. Cytogenetically ambiguous results caused by factors such as maternal cell contamination or culture-related mosaicism are reported more often after CVS than after amniocentesis[6]. However, ambiguous CVS results also may indicate a condition (e.g., confined placental mosaicism) that has been associated with adverse outcomes for the fetus[7,8]. Thus, in these situations, CVS may be more informative than amniocentesis alone.

Chorionic villus sampling (CVS) is a technique for retrieval of fetal cells from developing pregnancy during the first trimester. The transcervical approach, the first to be used clinically in Europe and Northern America, employs the use of a flexible catheter inserted through the cervix into the developing placenta with subsequent suction removal of a small amount of villi[9,11]. In 1984, the alternative transabdominal approach was introduced[12]. This technique, which utilizes a stiff needle inserted through the abdominal wall into the developing placenta, offered theoretic benefits of lowered risk of infection, higher patient acceptability, and greater ease of learning because of its similarity to amniocentesis. In Thailand, most women who are at risk for having a chromosome abnormality fetus will be advised to have second-trimester amniocentesis. The authors have been using chorionic villus sampling technique since November 10, 1997 and the authors hope their experience will encourage the use of this technique in Thailand.

Material and Method

From November 10,1997 to October 17,2006, 383 women were recruited, twenty-two patients were excluded because ultrasound examination showed an embryonic pregnancy or fetal demise *in utero* (13 cases), multiple fibroid (4 cases), large areas of placental hemorrhage (3 cases), in appropriate uterine position (1 case) and 1 case of multiple pregnancy. The authors had 361 cases of chorionic villus sampling. On only 2 of 361 cases CVS were performed transcervically. The most common indication for chorionic villus sampling was advanced maternal age. After a process of counseling and signing informed consent forms, a detailed ultrasound examination was performed to confirm gestational age, viability, placental position and to exclude multiple pregnancy. Sampling was carried out mostly between 10-12 weeks of pregnancy. For abdominal approach, chorion frondosum was located with curvilinear probes. To secure the predetermined needle pathway, a needle guide was mounted on the transducer. The needle pathway was optimal when established as a line parallel to the chorionic membrane. The situation was often optimal when the bladder was nearly empty. Moreover, it was not necessary to await relaxation of any uterine contraction. The double needle biopsy system consists of a coaxial guide needle gauge 18, length 15 cm, and a sampling needle gauge 22, length 20 cm, the biopsies were aspirated through a combined cutting and suction exerting 10 ml vacuum. The sampling took place as an outpatient procedure, with an average time consumption of 10 minutes. All of the procedures were under aseptic technique.

For transcervical technique, after scanning, the patient was placed in the lithotomy position the vulva and the perineal area were cleansed thoroughly with povidone-iodine solution and sterile drapes placed covering this area except the vaginal introitus. A bivalve speculum was gently introduced. Then the cervix and vagina were cleansed thoroughly with povidone-iodine solution. The cannula was gently passed through the cervix, under ultrasound guidance, until a loss of resistance was felt at the endocervix, 10 ml syringe with tissue media was applied to the cannula, and negative pressure was applied during moving the cannula along the placenta site. The patient was allowed to return home 30 minutes after the procedure, and ultrasound examination was arranged during 18-20 weeks of pregnancy to confirm anomalies and sex. The results were expressed as the frequency and percentage in the frequency distribution tables.

Results

A total of 361 cases of chorionic villus sampling were performed from November 10, 1997 to October 17, 2006. Of these, 359 women had transabdominal CVS and 2 women transcervical CVS as shown in Table 1. Second trimester amniocentesis following CVS was required in 10 cases (2.77%) due to maternal cell contamination, failed tissue culture and mosaicism. Most of the indications for CVS were elderly gravida as shown in Table 2. The distribution of the women's age is shown in Table 3. Three hundred and forty-six cases (95.84%) were more than 35 years of age. The most common age group were 35-39 year olds (82%) as shown in Table 3. All women aged under 35 years were due to abnormal ultrasound findings, abnormal Thalassemia screening and previous Down syndrome. The youngest in age was 24 years and the oldest 44 years. Nearly 100% of CVS was done by transabdominal technique and the most common gestational age was 10-12 week of pregnancy. The authors had only 1 case done at 17 weeks of pregnancy due to severe oligohydramnios as

Table 1. Number of chorionic villus sampling per year

Year	CVS		Total
	Transabdomen	Transcervical	
1997	9	0	9
1998	56	2	58
1999	88	0	88
2000	70	0	70
2001	35	0	35
2002	20	0	20
2003	24	0	24
2004	23	0	23
2005	19	0	19
2006	15	2	15
Total	359	2	361

Table 2. Indications for chorionic villus sampling

Indications	Cases	%
1. Elderly gravidarum	346	95.84
2. Abnormal Thalassemia screening	6	1.60
3. Abnormal ultrasound	3	0.83
4. Family chromosome disorder	3	0.83
5. Previous Down syndrome	2	0.56
6. Severe oligohydramnios	1	0.28
Total	361	100.00

Table 3. Distribution of maternal age

Ages (years)	Cases	%
20-24	2	0.6
25-29	4	1.1
30-34	9	2.5
35-39	299	82.8
40-44	47	13.0
Total	361	100.0

shown in Table 2. Cytogenetic results of chromosome abnormalities are shown in Table 4. The authors successfully obtained sufficient chorionic tissue in all of the cases. From 351 women, a sufficient amount of villi were aspirated at the first attempt. An attempt included insertion of the guide needle and 2-4 repeated aspirations through the guide needle. The average period of guide needle insertion was 5 minutes.

The authors had 4 cases of maternal cell contamination, 3 cases of failed tissue culture (2 in transcervical technique and 1 in transabdominal technique) and two cases of mosaicism. All of them were normal chromosome studies after performing second trimester amniocentesis. The authors had two fetal losses, one from 47xx+7 and the other from 46xx. In the present study the authors had few non-serious complications after transabdominal CVS, 2 cases of minimal vaginal bleeding and 2 cases of abdominal pain. The cytogenetic accuracy rate was 97.50%.

Discussion

In the present study, 359 diagnostic transabdominal CVS and 2 transcervical CVS were performed by only one operator. The sampling efficiency was 100% is slightly higher than the previous report[13]. Although the data appeared to confirm that the two techniques are equally effective in obtaining an adequate amount of chorionic tissue, transabdominal needling has a statistically significant smaller proportion of repeated device insertions (3.3% versus 10.3%). Although the number of insertions does not appear to influence immediate complications or pregnancy outcome, repeated maneuvers are certainly time-consuming, as well as possibly being uncomfortable for the woman, and this finding thus seems to explain, at least partly, the authors preference for the transabdominal technique[14,15]. With an empty bladder and increased transducer pressure against the abdominal wall, sampling was possible in almost all cases. The stabbing of the abdominal wall and the uterus must be exerted quickly and without hesitation, first to reduce discomfort and secondly to secure the correct position of the guide needle, which has to make contact with uterine surface in a way that secures immediate uterine perforation and fixation. The correct needle contact will prevent it sliding on the uterus.

A major concern with all prenatal diagnostic technique is the possibility of discordance between the prenatal cytogenetic diagnosis and the actual fetal karyotype. This occurs, though infrequently, following amniocentesis shown by Loft and Tabor who estimated that the cytogenetic accuracy rate ranges from 99.2% to 99.9%[16], and 100% from transabdominal CVS[17]. From the present series the cytogenetic accuracy rate was only 97.50%, which was lower than previous reports due to maternal cell contaminations and failed tissue culture. The absolute fetal loss rate for chorionic villus sampling has ranged from 3.12%[2] to 3.3%[17] but the present study had only 2 fetal losses

Table 4. Chromosome abnormalities

Cases	Karyotype		Outcome	Maternal age
	CVS	Amniocentesis		
1	46xx, 46xy	46xy	normal male	36
1	46xx, 16p+	46xx, 16p+	normal female	40
1	46xy, 46xx	46xy	normal male	35
1	47xx, +7		spontaneous abortion	36
1	47xy, +13		termination	38
1	46xx, 48xx+21, +21 (1:1)	46xx	normal female	35
1	47xxx, 46xx	46xx	normal female	38
2	47xy, +18		termination	37, 40
4	47xy, +21		termination	42, 40, 40, 40
1	46xy, 46xx (1:6)	46xy	normal male	36
1	47xy+13		termination	34
1	46xx, 46xy (1:1)	46xy	normal male	38
1	45xo		spontaneous abortion	29

(0.5%), one from 47xx+7 and the other from normal karyotype. The frequency of mosaicism was found between 1%[8,18] and 1.7%[19] but the present study found 0.5%. Firth et al[20] reported 5 babies with severe limb abnormalities among 289 pregnancies in which CVS was carried out at 56-66 days of gestation. Population-based studies indicate that the risk for all limb deficiencies is from 5-6 per 10,000 live births[21]. Investigators participating in the International Registry also have combined birth-defect data from multiple CVS centers, including some of 65 CVS centers[22,23]. An abstract published in 1994 includes information about 138,000 procedures reported to the International Registry. The rate of transverse deficiencies in the reporting centers was 1.4 per 10,000 procedures, lower than most population-based rates. In the present study, no case of limb reduction defect was found. Earlier concerns about procedure-induced limb defects have been reduced with the accumulation of additional data showing minimal to no risk when CVS is performed after 70 days of gestation[24]. The worldwide most common indication for fetal karyotyping was maternal age as the same as this report.

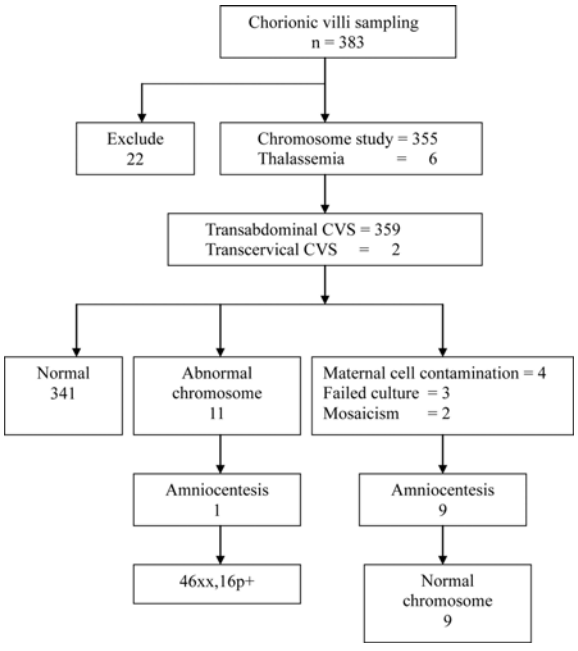

Fig. 1 Diagnostic procedures and outcome of investigations

In conclusion, the authors have found that early prenatal diagnosis by transabdominal chorionic villus sampling had few complications and did not threaten maternal health. Transabdominal CVS seems to cause no more serious complications than those after amniocentesis, except increasing rate of maternal cell contamination and mosaicism. The sampling success rate is high. During the procedure, the women rest relaxed in the supine position. The slight discomfort is quite the same as amniocentesis. Studies comparing very experienced practitioners, (more than 100 procedures per annum) with much less experienced practitioners have shown substantial differences in outcome, with six- to eight-fold increases in loss rates by the less

experienced doctors[25-28]. At this time, early amniocentesis cannot be assumed to be equal to conventional transabdominal CVS or amniocentesis with regard to safety or accuracy[29].If early diagnosis is required, transabdominal CVS is preferable to early amniocentesis or transcervical CVS[30]. For safety reasons the authors suggest that transabdominal CVS should be performed by experienced operators. The authors believe that transabdominal CVS by experienced operators is an alternative procedure to amniocentesis in the detection of genetic disorders and Thalassemia disorders in early pregnancy in Thailand.

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การตรวจเนื้อรกเพื่อวินิจฉัยความผิดปกติของทารกในครรภ์ช่วงไตรมาสแรกในโรงพยาบาลภูมิพลอดุลยเดช

วิบูลย์ เรืองชัยนิคม, สราวุธ สารภักดิ์, นวภรณ์ ออรุ่งโรจน์

วัตถุประสงค์*: เพื่อศึกษาผลลัพธ์ของการตรวจเนื้อรกเพื่อการวินิจฉัยความผิดปกติของทารกในครรภ์ ในโรงพยาบาลภูมิพลอดุลยเดช*

วัสดุและวิธีการ*: ศึกษาในหญิงตั้งครรภ์ จำนวน 383 ราย อายุครรภ์ระหว่าง 10-12 สัปดาห์ ตั้งแต่วันที่ 10 พฤศจิกายน พ.ศ. 2540 ถึงวันที่ 17 ตุลาคม พ.ศ. 2549 ที่หน่วยดูแลสุขภาพทารกในครรภ์ โรงพยาบาลภูมิพลอดุลยเดช โดยหญิงตั้งครรภ์ทุกรายจะได้รับการตรวจอุลตราซาวนด์หาอายุครรภ์ ตำแหน่งรก และความผิดปกติของทารก และจะได้รับคำอธิบายถึงผลดีและผลเสียของการตรวจเนื้อรกจนเป็นที่เข้าใจ ก่อนที่จะเซ็นยินยอมและได้รับการ ตรวจเนื้อรก หลังจากนั้นจะมีการติดตามภาวะแทรกซ้อนจากการตรวจเนื้อรกอีกครั้งช่วงอายุครรภ์ 18-20 สัปดาห์ พร้อมตรวจอุลตราซาวนด์ ดูเพศ และความผิดปกติของทารกในครรภ์*

ผลการศึกษา*: หญิงตั้งครรภ์ที่ได้รับการตรวจเนื้อรกทั้งสิ้นมีจำนวน 361 ราย โดยได้รับการตรวจเนื้อรกผ่านทางหน้าท้อง 359 ราย ตรวจเนื้อรกผ่านทางช่องคลอด 2 ราย 22 ราย ไม่ได้รับการตรวจเนื่องจากพบทารกเสียชีวิตก่อนตรวจ 13 ราย มีเนื้องอกมดลูก 4 ราย มีเลือดออกชั้นใต้รก 3 ราย ครรภ์แฝด 1 ราย และอีก 1 ราย ไม่สามารถหาตำแหน่งของรก ที่เหมาะสมได้ ข้อบ่งชี้ของการตรวจที่พบมากที่สุด ได้แก่มารดาอายุเท่ากับ หรือ มากกว่า 35 ปี เมื่อครบกำหนดคลอด คิดเป็นร้อยละ 95.84 ตรวจพบโครโมโซมผิดปกติ 11 ราย เนื้อเยื่อมารดาปนเปื้อน 4 ราย, เพาะเลี้ยงเนื้อเยื่อไม่ได้ 3 ราย และพบ mosaicism 2 ราย ผู้ป่วยได้รับการตรวจน้ำคร่ำซ้ำ 10 ราย คิดเป็นร้อยละ 2.77 ทารกเสียชีวิต จากการตรวจ 2 ราย โดย 1 ราย เป็น 47xx,+7 อีก 1 ราย เป็น 46xx*

สรุป*: การตรวจเนื้อรกเพื่อหาความผิดปกติของโครโมโซมและธาลัสซีเมียของทารกในช่วงไตรมาสแรกของการตั้งครรภ์น่าจะเป็นอีกทางเลือกที่ใช้ในการตรวจหาความผิดปกติของทารกในครรภ์ โดยพบภาวะแทรกซ้อนจากการ ทำไม่สูงมาก และยังช่วยในการวินิจฉัยได้เร็วกว่าการตรวจน้ำคร่ำ*