Chorionic Villous Sampling: Experience of 636 Cases

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Objective: *To describe the technical aspects and complications of chorionic villous sampling (CVS).*
Material and Method: *The database of CVS procedures performed between January 2004 and August 2011 at Maharaj Nakorn Chiang Mai Hospital was assessed prospectively. Consecutive procedures during 10 to 14 gestational weeks were included into the present study. Indications, results, and complications of the CVS were extracted and analyzed.*
Results: *All 636 CVS procedures were successful. Indications for CVS were fetal karyotyping, DNA analysis for severe thalassemia disease, and for both in 36.5%, 50.6%, and 12.9%, respectively. However, 3.4% had inconclusive CVS result, and the second trimester prenatal diagnosis procedures needed to be done. There were five cases (0.8%) of fetal loss in the present study. Only two cases (0.3%) that fetal loss happened within two weeks of CVS procedures. Other minor complications such as vaginal bleeding and amniotic fluid leakage were found in 0.3 to 1.3% and had no long-term effect. No case with anomaly and procedure-related infection following the procedure was seen.*
Conclusion: *CVS is a safe and reliable prenatal diagnosis procedure in the first trimester. In experienced operators, fetal loss rate was less than 1%. However, a few cases will have inconclusive CVS results and may need a confirmation during the second trimester diagnostic procedures.*

Keywords: *Chorionic villous sampling, Prenatal diagnosis, Fetal loss*

J Med Assoc Thai 2013; 96 (4): 383-8
Full text. e-Journal: *http://jmat.mat.or.th*

Over the past decades, chorionic villous sampling (CVS) has emerged as an alternative to amniocentesis for women who wish to have an early prenatal diagnosis. In addition, due to an increase in a number of pregnancies with advanced of maternal age and availability of first trimester aneuploidy screening program, demand of prenatal diagnosis procedure during the first trimester is increased accordingly. CVS is a procedure that retrieves cells from the developing placenta for genetic or chromosome analysis during first trimester. There are two approaches of CVS depending on placental accessibility, transabdominal and transcervical, of which safety and diagnostic accuracy are comparable[1].

The main advantage of CVS over amniocentesis or cordocentesis is that the definitive diagnosis could be made earlier in gestation. However, an invasive procedure carries a risk of pregnancy loss varying from 0.6 to 3% and needs more expertise[2-6]. Although there has been concern that CVS may lead to limb reduction defects, current data suggest that CVS performed between 10 to 14 weeks of gestation is not significantly associated with a higher incidence of such a condition[7,8]. However, if CVS was done before 10 weeks of gestation, the risk of limb reduction defect may probably increase to 1 to 2%[9,10]. The aim of the present study was to describe the experience of transabdominal and transcervical CVS at Maharaj Nakorn Chiang Mai Hospital, in the aspects of indications, success rate, and complications of the procedure.

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Material and Method

The present retrospective descriptive study recruited consecutive cases of singleton pregnant women with indication for CVS at 10 to 14 weeks gestation at Maharaj Nakorn Chiang Mai Hospital, between January 2004 and August 2011. All procedures, either by transabdominal or transcervical approach, were performed with informed consent. The six operators who performed CVS in the present study were familiar with ultrasound guided prenatal diagnostic procedures and had performed over 100 cases of cordocentesis before performing CVS. For transabdominal CVS, the procedure was performed

under ultrasound guidance, supine position under local anesthesia, using 20 gauge spinal needle with back and forth movement technique. For transcervical approach, the patient was placed in lithotomy position and the procedure was performed using Rodeck CVS biopsy forceps under continuous ultrasound guidance. Once obtained, the sample was immediately examined under a microscope to determine if the amount of villi were adequate and remove the decidua (maternal cells) from the villi. Then the tissues were sent to laboratories for processing. For karyotyping, the chorionic villi were cultured and analyzed. For DNA analysis, extraction of DNA was performed and PCR-base technique was used for mutation detection. Success rate of procedures, pregnancy outcomes, and procedure related complications were prospectively assessed and recorded. The data were analyzed using the statistical package for the social science (SPSS, Chicago), version 15.0. Descriptive data were presented as percentage, mean, and standard deviation (SD) and 95% confidence interval (95% CI). The present study was approved by the Institutional Review Boards (IRB) of ethics committee and financially supported by the National Research University Project under Thailand's office of the Higher Education Commission.

Table 1. Results of CVS

Results	Cases (%)
Karyotype	314 (100)
Normal	275 (87.6)
Autosomal trisomy (trisomy 21, 18, 13, 7)	15 (4.8)
45,XO	5 (1.6)
Mosaicism	3 (1.0)
Others	4 (1.2)
No results due to laboratory failure	12 (3.8)
Severe thalassemia	404 (100)
Normal	86 (21.3)
Trait	203 (50.2)
Affected	109 (27.0)
No results due to laboratory failure	6 (1.5)

Results

During the study period, 636 pregnancies were recruited into the study. The mean maternal age was 32.3±5.5 years, and the mean gestational age was 12.1±0.9 weeks. Ninety two point five percent (588 cases) and 7.5% (48 cases) had transabdominal and transcervical CVS, respectively. The procedure was successful in all cases, and 89.6% (570 cases) were success with one attempt of puncture. Only four cases (0.6%) needed three or more punctures to retrieve adequate samples. The indications for CVS were fetal karyotyping, DNA analysis for severe thalassemia diseases and for both karyotype and DNA analysis in 36.5% (232 cases), 50.6% (322cases), and 12.9% (82 cases), respectively. However, the final results could not be obtained in 3.1% (20 cases), consisting of two cases (0.3%) with maternal cell contamination and 18 cases (2.8%) with problems in laboratory techniques including inadequate metaphase for karyotyping (12 cases, 1.9%), unable to identify globin gene mutation (5 cases, 0.8%), and inconclusive result for DNA analysis of severe thalassemia disease in one case (0.1%).

Table 2. Details of cases with abnormal karyotypes from CVS (n = 27)

CVS results	Number (cases)	US findings	Second trimester PND	Pregnancy outcome
Trisomy 21	6	Thick NT all	-	Therapeutic abortion
Trisomy 18	6	Thick NT all	-	Therapeutic abortion
Trisomy 13	2	Thick NT all	-	Therapeutic abortion
Trisomy 7	1	Normal US	46,XX	Normal female newborn
45,XO	4	Cystic hygroma	-	Therapeutic abortion
45,XO	1	Normal US	46,XX	Normal female newborn
46,XX/47,XXY	1	Normal US	46,XY	Normal male newborn
46,XY/47,XX +13	1	Normal US	46,XY	Normal male newborn
46,XY/47,XX +7	1	Cystic hygroma	-	Therapeutic abortion
47,XXY	1	Normal US	-	Therapeutic abortion
47,XYY	1	Normal US	-	Normal male newborn
46,XX, del (22) (q11.2)	1	Normal US	46,XX, del (22) (q11)	Normal female newborn
46,XY, inv (6)	1	Normal US	46,XY	Normal male newborn

The results of CVS are shown in Table 1. Abnormal karyotypes from CVS results were found in 8.6% (27 cases) and details of individual case are presented in Table 2.

Twenty-two cases (3.4%) needed further prenatal diagnosis procedures in the second trimester to clarify the CVS results. The reasons for confirmatory tests were failure in laboratory techniques (14 cases; 2.2%), maternal cell contamination (2 cases; 0.3%), and abnormal karyotypes without any sonographic soft signs or structural abnormalities (6 cases; 0.9%). Among these six cases, one case with microdeletion of chromosome 22, which was confirmed by cordocentesis, was proven to be inherited from the mother and the pregnancy outcome was uneventful. In contrast, the other five cases had normal karyotype from cordocentesis, though CVS showed abnormal results. Additionally, 27.0% (109 cases) of fetuses at risk of severe thalassemia diseases were identified as affected fetuses by CVS. There were 11.6% (47 cases), 11.4% (46 cases), and 4.0% (16 cases) of fetuses with homozygous alpha thalassemia-1 (Hb Bart's), beta-thalassemia/Hemoglobin E disease, and homozygous beta-thalassemia diseases, respectively. However, CVS could not diagnose for thalassemia status in 1.5% (6 cases) due to failure in laboratory techniques as shown in Table 1.

The complications of CVS are shown in Table 3. There were missing data in 22 cases (3.4%). Therefore, 614 cases were left for analysis. Five cases (0.8%) had fetal loss. Of them, two fetal losses occurred within two weeks after the transabdominal CVS procedures, which were most likely to be procedure-related fetal loss. The first case had two attempts of CVS puncture but there was failure in laboratory technique due to inadequate metaphase of villi and the other case was fetal trisomy 18. However, there were three cases that fetal loss occurred more than two weeks after CVS, which were two cases of transabdominal and one case of transcervical approach and puncture in one attempt for all cases. Fetal loss occurred at 17, 24, and 16 gestational weeks, respectively. Both abortuses had no gross anomaly and CVS results showed normal in one case and thalassemia trait in the other two cases. Additionally, there were some minor complications following CVS procedure such as minimal vaginal bleeding that found in 1.3% (8 cases), which most of them were transcervical CVS. Amniotic fluid leakage also found in 0.3% (2 cases), the leakage occurred within a few days after procedure and ceased spontaneously, both of them had normal term

Table 3. Complications of CVS

Complications	Transabdominal CVS 568 cases (missing data 20 cases) n (%) (95% CI)	Transcervical CVS 46 cases (missing data 2 cases) n (%) (95% CI)	Total 614 cases (missing data 22 cases) n (%) (95% CI)
Fetal loss within 2 weeks after CVS	2 (0.4) (-0.12 to 0.92)	-	2 (0.3) (-0.13 to 0.73)
Fetal loss more than 2 weeks after CVS	2 (0.4) (-0.12 to 0.92)	1 (2.2) (-2.04-6.44)	3 (0.5) (-0.06 to 1.06)
Vaginal bleeding	3 (0.5) (-0.08 to 1.08)	5 (10.9) (1.89-19.91)	8 (1.3) (0.4 to 2.2)
Amniotic fluid leakage	2 (0.4) (-0.12 to 0.92)	-	2 (0.3) (-0.13 to 0.73)
Small for gestational age	12 in 447 cases (2.7) (1.2-4.2)	-	12 in 480 cases (2.5) (1.1 to 3.9)
Deliver before 34 weeks	6 in 447 cases (1.3) (0.25-2.35)	1 in 33 cases (3) (-2.82-8.82)	7 in 480 cases (1.5) (0.41 to 2.59)
Deliver between 34-36 weeks	29 in 447 cases (6.4) (4.13-8.67)	2 in 33 cases (6) (-2.1-14.1)	31 in 480 cases (6.5) (4.29 to 8.71)

newborns. There was no procedure-related infection in the present study. Therapeutic abortion due to abnormal chromosome, severe thalassemia disease, and severe fetal anomaly was carried out in 134 (21%) cases. Therefore, 480 cases (447 cases of transabdominal and 33 cases of transcervical CVS) were left for analysis of long-term complications. In assessment of newborns, no case of structural defect was found to be related to the CVS procedures. The prevalence of newborns with small for gestational age was only 2.5% (12 cases) and prevalence of preterm delivery was 8.0% (38 cases), which only 1.5% (7 cases) that delivered before 34 weeks, both of these events were unlikely to be affected by CVS procedure. Based on the outcomes, using clinical estimation of the newborns, no cases of severe thalassemia or abnormal major chromosomal abnormalities were missed.

Discussion

CVS is one of the prenatal diagnosis procedures that have been widely accepted for more than decades. Many publications have been reported about its safety and accuracy of the result[8,11,12]. CVS-related fetal loss was reported in the range of 0.6-3%[5,6,8,11,13,14] and incidence of confined placental mosaicism was 1 to 2%[15].

From the 636 CVS procedures in the authors' study, most of the procedures were transabdominal approach. The procedure was successful in all cases, which almost 90% were successful in one attempt of transabdominal puncture or using Rodeck CVS biopsy forceps in transcervical cases. Most cases that required more than one puncture were usually due to the difficulty to access placental location or the period of operators' learning curve in performing CVS. DNA analysis for severe thalassemia disease was the most common indications for CVS in the present study with the benefit of early diagnosis and less procedure-related fetal loss compared to cordocentesis during mid-pregnancy[15,16]. However, due to limitations and failure in laboratory techniques, data from the present study found that CVS results could not be obtained in around 3% of the cases, which is consistent with other studies[17,18]. This is one important information that should be given to patients about limitation of CVS procedure.

Few cases also need confirmatory prenatal diagnosis procedures due to inconsistent between CVS results and ultrasound findings. Most of these cases had abnormal karyotype from CVS such as autosomal trisomy, Turner's syndrome, mosaicism, deletion of chromosome and chromosomal inversion. However, all of these cases had normal ultrasound findings, which were unlikely if the CVS results were correct. Therefore, it was reasonable to repeat the prenatal diagnosis procedure to confirm the abnormalities of fetal karyotype in such a circumstance. Finally, either amniocentesis or cordocentesis in these cases showed normal karyotype in most of them, except for one case with microdeletion of chromosome 22 that was inherited from the mother. Thus, interpretation of CVS results should be done together with ultrasound findings. Not only mosaicism from CVS results that should be repeated to exclude confined placental mosaicism, but also in case of abnormal karyotype by CVS with no fetal structural defects. Therefore, after excluding five cases of normal chromosome after repeat testing, the rate of true abnormal chromosome in the present study was 7.0%. Moreover, in the aspect of DNA analysis in fetuses at risk of severe thalassemia diseases, CVS cannot identify gene mutation in 1.5% of the cases and cordocentesis for fetal hemoglobin typing need to be done during mid-pregnancy. Therefore, this data is also an important issue for counseling the patients about the limitation of CVS in case of fetal at risk for severe thalassemia diseases. Of note, such a problem was not associated with CVS itself but rather laboratory technique, signifying that improvement in laboratory quality control may be more important than CVS techniques.

From the result, total fetal loss rate from CVS is 0.8% (5 cases), which is lower than previous studies[3,4] and comparable to fetal loss rate from second trimester amniocentesis[11]. Moreover, only two in five cases of fetal loss occurred within two weeks after CVS, which defined as procedure-related fetal loss. In addition, one of these two cases was trisomy 18 fetus, which might have been the cause of fetal loss rather than procedure-related. Therefore, the true rates of CVS-related fetal loss in the present study might possibly be only 0.2 to 0.3%. The reason that fetal loss rate in the present study is very low may be related to experience of the operators and most of the cases in the present study are transabdominal CVS, which is relatively safer than transcervical approach. All operators who performed CVS in the present study were familiar with ultrasound guided prenatal diagnostic procedures such as amniocentesis and cordocentesis, and all of them had performed over 100 cases of cordocentesis before performing CVS. In the aspect of other complications, incidence of minor complications such as leakage of amniotic fluid

or vaginal bleeding were around 0.3 to 1.3% and had no long-term effect on the fetuses. Vaginal bleeding is the complication that was common in transcervical approach. CVS-related fetal anomaly as limb reduction defect, and infection after the procedures were not found in the present study. The incidences of small for gestational age babies and preterm birth were only 2.5% and 8.0%, respectively, which are very low and unlikely to be the result from CVS procedure.

With the limitation of descriptive study, the authors did not have the control group to compare the complications especially fetal loss rate and long-term effect of the CVS procedure. However, the present study had a large sample size of more than 600 cases of CVS and included almost 50 cases of transcervical CVS. Moreover, the study population is homogenous and confined within the period of 10 to 14 gestational weeks, which are the most safe and common periods of performing CVS. Therefore, the information from the present study is valid and may be useful in clinical practice.

Conclusion

The present study suggests that both transabdominal and transcervical CVS are relatively safe and reliable methods of prenatal diagnosis in late first trimester. In experienced hands, fetal loss rate is less than 1% and no long-term complications for fetuses. However, a minority of cases with inconclusive CVS results may need a confirmation by second trimester diagnostic procedures.

Acknowledgement

The authors wish to thank the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

Potential conflicts of interest

None.

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การตัดชิ้นเนื้อรก: ประสบการณ์ 636 ราย

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วัตถุประสงค์: *บรรยายเทคนิคและภาวะแทรกซ้อนจากการตัดชิ้นเนื้อรก*
วัสดุและวิธีการ: *วิเคราะห์ฐานข้อมูลการตัดชิ้นเนื้อรกในช่วงอายุครรภ์ 10-14 สัปดาห์ ของโรงพยาบาลมหาราชนครเชียงใหม่ ตั้งแต่มกราคม พ.ศ. 2547 ถึงสิงหาคม พ.ศ. 2554 โดยประเมินเกี่ยวกับข้อบ่งชี้ผลลัพธ์และภาวะแทรกซ้อน*
ผลการศึกษา: *สตรีตั้งครรภ์ 636 ราย ข้อบ่งชี้ในการตัดชิ้นเนื้อรกคือตรวจโครโมโซมทารก, ตรวจโรคธาลัสซีเมีย และตรวจทั้งสองชนิด คิดเป็นร้อยละ 36.5, 50.6 และ 12.9 ตามลำดับ อย่างไรก็ตามมีร้อยละ 3.4 ที่สรุปผลไม่ได้จากการตัดชิ้นเนื้อรก ทำให้ต้องวินิจฉัยก่อนคลอดอีกครั้งในช่วงไตรมาสที่สอง พบว่ามีทารกที่แท้งหลังการเจาะร้อยละ 0.8 โดยมีเพียงร้อยละ 0.3 ที่การแท้งเกิดขึ้นภายใน 2 สัปดาห์ เลือดออกหรือน้ำเดินพบได้ร้อยละ 0.3-1.3*
สรุป: *การตัดชิ้นเนื้อรกมีความปลอดภัย ถ้าทำโดยแพทย์ผู้ชำนาญจะมีการแท้งน้อยกว่าร้อยละ 1 อย่างไรก็ตามอาจมีผู้ป่วยจำนวนหนึ่งที่ต้องรับการวินิจฉัยก่อนคลอดด้วยวิธีอื่นซ้ำ เนื่องจากผลของการตัดชิ้นเนื้อรกไม่ชัดเจน*
