

Sh. Akhlaghpoor MD¹
A. Aziz Ahari MD²

Chorionic Villus Sampling and Marked Membrane Separation

Background/Objective: The major concern about the invasive prenatal diagnostic tests is the frequency of procedure induced pregnancy loss. Chorionic Villus Sampling (CVS) is the invasive test of choice in the first trimester after the 10th gestational week. Our experience suggests marked chorioamniotic separation is an uncommon finding after the 10th gestational week. This study assesses the rate of marked membrane separation in a 10 to 14-week gestational period and its effect on post CVS fetal loss.

Patients and Methods: Forty-one patients (5.2%) were selected among 782 patients as cases with marked membrane separation (mean maternal age, 26.9 years). CVS procedures were performed with a 20-gauge Chiba needle attached to a 20-ml syringe under ultrasound guidance. Follow-up was performed by phone call and clinical visits until 24 weeks of gestation. For the control group, the follow-up was performed for only 2 weeks. Early fetal loss in the first two weeks of post procedural period, and late fetal loss from 2 weeks after procedure till the 24th gestational week were considered as CVS complications.

Results: We detected 2.4% early fetal losses after the procedure. Fourteen cases voluntarily underwent therapeutic abortion due to beta-thalassemia or hemophilia. One fetus with microcephaly was spontaneously aborted in the 21st gestational week. Twenty-five neonates were delivered alive at term and one prematurely at the 32nd week. Marked membrane separation had no significant effect on early post CVS fetal loss rate.

Conclusion: The procedure does not have a major impact on the early post CVS fetal loss in patients with marked membrane separation.

Keywords: Chorionic Villus Sampling, Fetal Loss, Prenatal Diagnosis

Introduction

The first reports of invasive prenatal diagnosis were in the 1960s^{1,2} and many indications have been postulated for their use so far. Regarding the most recent American College of Obstetricians and Gynecologists' (ACOG) Practice Bulletin in January 2007, all pregnant women, regardless of their age or reason, should have the opportunity to opt for diagnostic testing via Chorionic Villus Sampling (CVS) or amniocentesis.³

Concerning the potential risks of such procedures, patients and clinicians should be aware of a spectrum with two extremes of potentially bad outcomes; at one end, a chance of a baby with a serious genetic disorder, and at the other end, the chance of losing an otherwise normal and wanted pregnancy. Many papers have been published on the timing and real risks and benefits of CVS and amniocentesis which are mostly used in prenatal invasive diagnostic tests.⁴⁻⁶ However, the frequency of procedure-induced pregnancy loss makes up the major concern about prenatal diagnostic procedures.⁴

CVS was first introduced in China in the mid-1970s,⁷ then the procedure developed further worldwide. Unlike amniotic fluid aspiration performed in amniocentesis, this procedure requires aspiration of placental tissue. Percutaneous transabdominal or transvaginal/transcervical approaches are used under the guidance of ultrasound.⁸

1. Associate Professor, Department of Radiology, Novin Medical Radiation Institute, Tehran, Iran.

2. Resident of Radiology, Department of Radiology, Tehran University of Medical Sciences, Tehran, Iran.

Corresponding Author:
Shahram Akhlaghpoor
Address: Novin Medical Radiation Institute, 5th Ave., South Piroozan Ave. Hormozan St., Shahrak Ghods, Tehran, Iran.
Tel: +9821 8808 6783
Fax: +9821 8808 6782
Email: akhlaghpoor@nmri-ir.com

Received February 17, 2010;
Revised August 22, 2010;
Accepted December 23, 2010.

Iran J Radiol 2010; 7(4):211-214

At the present time, the selection between these approaches depends on the operator's personal preference,⁹ although some studies suggest higher risk in the transvaginal approach.¹⁰ Compared to the abdominal approach, the transvaginal approach needs considerably more skill and experience and it takes time for the operator to become competent in it.⁴ Moreover, it requires multiple and more frequent insertions, and causes vaginal bleeding in about 10% of the cases.⁸

In a recent meta-analysis on 16 studies for CVS, the loss rate within 14 days of the procedure, the loss rate before 24 weeks of gestation, and the total loss rate have been 0.7%, 1.3%, and 2.0%, respectively.⁵ CVS before the 9th gestational week may be accompanied with the increased incidence of fetal limb reduction defects.^{8,11} Currently, most clinicians use CVS as the invasive prenatal test of choice in the first trimester after the 10th gestational week.^{4,8}

Amnion and chorion membranes are physiologically separated during the first 12-13 weeks of pregnancy, and there is a fluid-filled extraembryonic cavity between them. The biochemical and hormonal features of amniotic and extraembryonic celomic fluids are different¹²⁻¹⁴ representing their compartment-specific bio-production.¹⁵ At 13-14 weeks, close juxtaposition of amnion and chorion membranes reduces the size of the celomic cavity to a virtual cavity.¹²

Although physiologic fusion of amnion and chorion membranes occurs before 14 weeks gestation¹⁶ and after this time, the detection of chorioamniotic separation should be considered abnormal,¹⁷ our experience shows even after the 10th gestational week (the least gestational age for performing CVS), marked chorioamniotic separation is an uncommon finding. The aim of this study was to report the rate of marked membrane separation in the 10th to 14th gestational week and to assess its effect on the frequency of post CVS fetal loss.

Patients and Methods

Between 2007 and 2009, forty one patients (5.2%) were selected among 782 patients referred for CVS for any indication as cases with marked membrane separation (i.e. more than 10 mm by our definition) (Fig. 1). The mean maternal age for all 782 patients was 27.1 years (range, 18-35 years). Table 1 summarizes the



Fig. 1. Two fetuses with marked membrane separation before CVS.

A. An 80-day fetus with a 12 mm membrane separation.

B. A 77-day fetus with a 13 mm membrane separation.

characteristics of the selected 41 patients. We excluded cases with gestational age of lower than 10 weeks, large sized subchorionic hematoma (greater than 50% of gestational sac),¹⁸ history of recurrent abortion, active gynecological infection and watery discharge. Our study had the institutional ethical board approval, and the informed consent was obtained from the patients.

All procedures were carried out after 10 weeks of gestation with regard to the crown-rump length and/or biparietal diameter measured immediately before the procedure. At first, transabdominal ultrasonographic evaluation was performed using a 3.5 MHz probe (Toshiba Just Vision, Toshiba, Japan) to detect any visible obstetric complication or contraindication for the CVS.

All transabdominal CVS procedures were performed with a 20-Gauge Chiba needle pass attached to a 20-ml syringe under continuous ultrasound guidance. The samples were immediately emptied into a disposable sterile plastic dish and flushed with normal saline. Then, they were assessed for an adequate amount

of villi on bright yellow light without any inverted microscope or loop. In case of no sufficient samples, the procedure was repeated up to three times. After the procedure, patients were monitored in a recovery room for 30 minutes and were discharged afterwards. The follow-up was performed by telephone and clinical visits until 24 weeks of gestation but for the patients without marked membrane separation, the follow-up was only for two post-procedural weeks.

Those patients who underwent therapeutic abortion or lost a fetus with a major anomaly (e.g. microcephaly) were excluded from analysis after the abortion. Early fetal loss in the first two weeks of post-procedural period and late fetal loss from 2 weeks after procedure until the 24th gestational week were considered as CVS complications.

Among the selected 41 patients, two cases had a small- and medium-sized subchorionic hematoma before the procedure which was not a contraindication for CVS regarding our exclusion criteria. The classification of hematoma was based on its size relative to the gestational sac size, and was categorized as small (less than 20%), medium (20–50%), or large (greater than 50%).

Early fetal loss occurrences in patients with and without marked membrane separation were compared with SPSS statistical software, version 16.0.

Results

In the selected cases, the gestational ages varied from 10 weeks and 0 day to 12 weeks and 5 days, and different placental positions were observed (Table 1). The overall success rate of single-needle-pass sampling was 95.1% (39 cases). For two patients (4.9%), the second-needle-pass sampling was performed, and there were no need for the third sampling in any patients. The mean chorioamniotic distance was 13.6 mm (range, 10-20 mm).

We detected one early fetal loss (one out of 41 patients, 2.4%) during the first two weeks after the procedure. This was a case with a medium-sized subchorionic hematoma. The volume of this hematoma was measured about 6 cc. The sampling for this patient was performed via a single needle pass.

After tissue sampling, histological study proved major beta-thalassemia in 13 cases (31.7%) and hemophilia in one case (2.4%). These cases voluntarily underwent

therapeutic abortion. One case had suspected microcephaly during ultrasound scanning for the procedure, and the anomaly was better visualized in the 19th week ultrasound exam. This fetus was spontaneously aborted in the 21st gestational week.

No late fetal loss was noted in the remaining 26 patients. Twenty-five neonates were delivered alive at term and one prematurely at the 32nd week. Among these 26 infants, four of the cases had minor beta-thalassemia and three were diagnosed with Down syndrome, neurofibromatosis and phenylketonuria (PKU).

The only measured data for the patients without marked membrane separation was the early fetal loss rate in two weeks after the procedure which was 1.48% (11 out of 741 patients). Chi-square showed no significant difference between the two groups of with and without marked membrane separation (cases and controls) in early fetal loss occurrence after CVS (P value = 0.37).

Table 1. Characteristics and Clinical Data of the 41 Selected Patients

Characteristics	Value
Number of patients	41
Gestational Age	76.1 ± 4.5 (1.5 weeks)
Mother's Age (year)	26.9 ± 4.3
Membrane Separation (mm)*	3 ± 2.
Placenta Location	
Anterior	18 (43.9%)
Posterior	19 (46.3%)
Fundal	4 (9.8%)

*Values are expressed as mean ± standard deviation.

Discussion

Our experience showed only 5.2% of the patients undergoing CVS have marked membrane separation, i.e. more than 10 mm by our definition. We assessed the frequency of post CVS fetal loss in the population with marked membrane separation. Only one patient (2.4%) experienced fetal loss (early) that was considered a procedural complication.

Based on our review of literature, we did not find any papers focused on the degree of chorioamniotic separation before the 14th week of gestational age. However, there were studies which reported different degrees of separation as abnormal chorioamniotic

separation after the 14th gestational week. Bronshtein et al.¹⁹ and Appelman et al.²⁰ took 10 mm, but Ulm et al.²¹ and Montero et al.¹⁷ took 3 mm as the cut-off. This limit was 1 mm for Levine et al.²² We defined the highest number (10 mm) as the limit for marked separation.

Unfortunately, the patients without marked membrane separation were only followed-up for two weeks and the only available data for this group was the rate of early fetal loss. So we could only statistically assess the effect of marked separation on early post CVS fetal loss. The data from a meta-analysis on prior studies showed the early, late and total post CVS fetal loss rates were 0.7%, 1.3%, and 2.0%, respectively.⁵

In the marked separation group, the only patient with fetal loss had a moderate-degree subchorionic hematoma at presentation. In this case, it is possible that the loss would be the impact of hematoma itself and not the CVS. It has been shown that small- and medium-sized subchorionic hematoma have no statistically significant effect on post CVS fetal loss, although the loss rate for the medium-sized one was reported higher.¹⁸ As mentioned before, there were no cases of late fetal losses in this group. Although the current study had a higher rate of early fetal loss in comparison with the previous studies, statistical analysis showed no significant impact of marked separation on early fetal loss.

In conclusion, this study shows that even before the 14th gestational week (10 to 14 week gestational week), marked separation of membranes is an uncommon finding. From the loss rates after CVS in case and control groups, we showed that the marked membrane separation does not have a major impact on early post CVS fetal loss.

Acknowledgment

The authors thank Mrs. Sodeh Mahabadi for her collaboration in data registry and follow-up of the patients.

References

1. Jacobson CB, Barter RH. Intrauterine diagnosis and management of

- genetic defects. *Am J Obstet Gynecol* 1967 Nov 15;99(6):796-807.
2. Nadler HL, Gerbie AB. Role of amniocentesis in the intrauterine detection of genetic disorders. *N Engl J Med* 1970 Mar 12;282(11):596-9.
3. Burgin KB. Prenatal genetic screening. *J Midwifery Womens Health* 2008 Jul-Aug;53(4):391-2.
4. Evans MI, Andriole S. Chorionic villus sampling and amniocentesis in 2008. *Curr Opin Obstet Gynecol* 2008 Apr;20(2):164-8.
5. Mujezinovic F, Alfrevic Z. Procedure-related complications of amniocentesis and chorionic villus sampling: a systematic review. *Obstet Gynecol* 2007 Sep;110(3):687-94.
6. Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986 Jun 7;1(8493):1287-93.
7. Department of Obstetrics and Gynecology, Tietung Hospital, Anshan, China. Fetal sex prediction by sex chromatin of chorionic villi cells during early pregnancy. *Chin Med J (Engl)* 1975 Mar;1(2):117-26.
8. Alfrevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2003;3:CD003252.
9. Alfrevic Z, von Dadelszen P. Instruments for chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2003;1:CD000114.
10. Smidt-Jensen S, Permin M, Philip J, Lundsteen C, Zachary JM, Fowler SE et al. Randomised comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling. *Lancet* 1992 Nov 21;340(8830):1237-44.
11. Brambati B, Simoni G, Travi M, Danesino C, Tului L, Privitera O et al. Genetic diagnosis by chorionic villus sampling before 8 gestational weeks: efficiency, reliability, and risks on 317 completed pregnancies. *Prenat Diagn* 1992 Oct;12(10):789-99.
12. Lewi L, Hanssens M, Spitz B, Deprest J. Complete chorioamniotic membrane separation. Case report and review of the literature. *Fetal Diagn Ther* 2004 Jan-Feb;19(1):78-82.
13. Itskovitz J, Rubattu S, Levron J, Sealey JE. Highest concentrations of prorenin and human chorionic gonadotropin in gestational sacs during early human pregnancy. *J Clin Endocrinol Metab* 1992 Sep;75(3):906-10.
14. Wathen NC, Cass PL, Kitau MJ, Chard T. Human chorionic gonadotropin and alpha-fetoprotein levels in matched samples of amniotic fluid, extraembryonic coelomic fluid, and maternal serum in the first trimester of pregnancy. *Prenat Diagn* 1991 Mar;11(3):145-51.
15. Jauniaux E, Gulbis B. Fluid compartments of the embryonic environment. *Hum Reprod Update* 2000 May-Jun;6(3):268-78.
16. Abboud P, Mansour G, Zejli A, Gondry J. Chorioamniotic separation after 14 weeks' gestation associated with trisomy 21. *Ultrasound Obstet Gynecol* 2003 Jul;22(1):94-5.
17. Montero JJ, Ortega S, Vazquez C, Martinez T, Loidi B. Delay of chorioamniotic fusion: relation to chromosomal anomalies. *Prenat Diagn* 2000 Jun;20(6):517-8.
18. Akhlaghpour S, Tomasian A. Safety of chorionic villus sampling in the presence of asymptomatic subchorionic hematoma. *Fetal Diagn Ther* 2007;22(5):394-400.
19. Bronshtein M, Zimmer EZ. Oligohydramnios with amnio-chorionic separation at 15-16 weeks' gestation. *Prenat Diagn* 1995 Feb;15(2):161-4.
20. Appelman Z, Zalel Y, Fried S, Caspi B. Delayed fusion of amnion and chorion: a possible association with trisomy 21. *Ultrasound Obstet Gynecol* 1998 Apr;11(4):303-4.
21. Ulm B, Ulm MR, Bernaschek G. Unfused amnion and chorion after 14 weeks of gestation: associated fetal structural and chromosomal abnormalities. *Ultrasound Obstet Gynecol* 1999 Jun;13(6):392-5.
22. Levine D, Callen PW, Pender SG, McArdle CR, Messina L, Shekhar A et al. Chorioamniotic separation after second-trimester genetic amniocentesis: importance and frequency. *Radiology* 1998 Oct;209(1):175-81.