

Chorionic villus sampling for beta-thalassemia: The first report of experience in Iran

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Objectives Beta-thalassemia is one of the most common hereditary disorders in Iran. The prenatal diagnosis of beta-thalassemia is part of a control program in our country and it began 13 years ago. During the past 8 years the number of procedures has increased significantly as also the legal abortions. This is the first report made on the CVS program in Iran.

Materials and Methods One thousand six hundred and sixty-one cases of transabdominal Chorionic Villus Sampling (CVS) have been retrospectively evaluated. Among them 1381 cases had inclusion criteria. CVS results, complications and fetal loss rate were evaluated. The distributions of the population at risk were divided between eight regions that have been proposed for beta-thalassemia mapping previously.

Results The mean age of the patients was 26.2 ± 5.2 years with mean gestational age of 11.4 ± 1.4 weeks. CVS was successful in all the patients (100%) although 1% required a second procedure. Post CVS fetal loss was 1.45%. Other minor complications were bleeding or spotting (1.81%), amniotic fluid leak (0.5%), small sub-chorionic hematoma (0.58%), severe abdominal pain (0.6%) and severe vasovagal reaction (0.14%). Late complications were seen in 0.21% (oligohydraminos). Approximately 2/3 of the patients were referred from three regions of the country, North (26.8%), South West (22.4%), Central (19.5%) and the remainder (31.3%) were from the other five regions.

Conclusion CVS is a safe and effective method for prenatal diagnosis of beta-thalassemia in countries with a high prevalence as in Iran. The overall complication rate is quite low and acceptable. Fortunately the recent acceptance of legal abortion with respect to Muslim rules has increased the effectiveness of the procedure and made great advances in its application in Iran. Correspondingly, social knowledge has also improved but still there is a gap between the population at risk and the required prenatal diagnosis laboratories and sampling centers. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: chorionic villus sampling; beta-thalassemia; prenatal diagnosis

INTRODUCTION

Beta-thalassemia is the most common autosomal recessive genetic disease in the Iranian population. More than two million carriers of beta-thalassemia live in Iran (Nozari *et al.*, 1995). It has been reported that its prevalence is about 5–10% (Karimi and Rasekhi, 2002). The birth rate of affected infants can be greatly reduced by screening to identify carriers, genetic counseling and prenatal diagnosis.

Iran started its national thalassemia prevention program in 1996. In the very first year the program aimed to identify carrier couples before marriage and to offer counseling, thus providing them with the opportunity to separate (Samavat and Modell, 2004). Prenatal diagnosis and the option of selective abortion were not widely available at that time.

Prenatal diagnosis has always been an option for couples at risk. Since prenatal diagnosis has become comparatively simple, recommendations are made for a community-based thalassemia control program (Alwan *et al.*, 1997).

For the implementation of a prenatal diagnosis program it is essential to know the spectrum of beta-thalassemia mutations in the population. Several studies have been conducted in Iran to show the common mutations in the Iranian population (Merat *et al.*, 1993; Noori-Dalooi *et al.*, 1994; Nozari *et al.*, 1995; Mahboudi *et al.*, 1996; Najmabadi *et al.*, 2001; Karimi and Rasekhi, 2002; Harteveld *et al.*, 2003). Most of these studies have been on a small number of patients or in specific areas. Najmabadi *et al.* (2001) have categorized national mutations widely so their study can be considered a basis for mutation study in Iran.

Unfortunately, no data have been reported on the Chorionic Villus Sampling (CVS) program in Iran. This is the first report of the largest CVS series in the country.

We report our experience with transabdominal CVS for prenatal diagnosis of haemoglobinopathies over 8 years and thus demonstrate the safety, efficacy and acceptability of CVS in Iran, a Muslim country.

MATERIALS AND METHODS

One operator carried out all the prenatal sampling procedures in a center. Since 1996 transabdominal CVS has been started in Iran. More than 2000 CVS have been

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carried out till June 2005. Unfortunately, due to the lack of standard registration, most of the first year's data, were not available for analysis.

From June 1999 until June 2005 (6 years), 1661 CVS cases were registered in our center; among them 1381 cases had the criteria to be entered in this retrospective study and 280 cases were excluded. Inclusion criteria were:

1. All available demographic data of the patient including place of birth.
2. Only Iranian cases were referred for thalassemia (excluding the patients from the neighboring countries).
3. Single gestational sac with gestational age ≤ 17 weeks.
4. Documentation of number of needle pass, placental location, any pre- or post-CVS events and complications.
5. Available follow-up for the procedure related fetal loss up to 2 weeks post CVS

The patients, were divided into eight groups based on regional divisions of the country, as reported by Najmabadi *et al.* (2001).

Five genetic laboratories in Tehran had referred the patients, and one each from the North and Southeast. All of these laboratories are active in prenatal diagnosis of thalassemia.

The mutations were characterized by a Polymerase Chain Reaction (PCR) method based on allele specific priming which is called amplification refractory mutation system (ARMS) (Najmabadi *et al.*, 2001).

Both parents were found to the carriers of beta-thalassemia and were informed by the obligatory premarital screening program for thalassemia or had given birth to a thalassemic major child. Parents were informed about the associated risks of the CVS, fetal abortion rates and were asked to sign a written informed consent. A baseline ultrasound investigation was performed. If any of the following conditions were found, the procedure was delayed:

1. Gestational age below 10 weeks (because of socio-economic problems some patients were not accepted; those who could not come later were also sampled at 9 weeks).
2. Normal ultrasound finding with active vaginal bleeding.
3. Visible subchorionic hematoma with recent spotting or bleeding.
4. Large significant asymptomatic subchorionic hematoma

In all the cases transabdominal CVS were performed with a 20 G Chiba needle, attached to a 20 cc syringe under free hand ultrasound guidance. The samples were immediately evacuated into a disposable sterile plastic dish and flushed with normal saline and then evaluated for adequate amount of villi using bright yellow light without any inverted microscope or loop. If the sample was not sufficient the procedure was repeated up to three times and if still not adequate the patient was planned for

another session the following week. After the procedure the patient was transferred to the recovery room and usually discharged after 30 min. The procedures were normally carried out as an outpatient procedure and if necessary they were hospitalized in case of any complication. The patient data was entered into the Microsoft Excel program (Microsoft Corp., USA) and analyzed.

RESULTS

The patients' age range was 16–40 years with a mean value of 26.2 ± 5.2 . The patients' gestational age range was 9–17 weeks with a mean value of 11.4 ± 1.4 . Almost 90% of the patients were between 10 and 13 weeks gestational age. Their frequencies are shown in Table 1. On the basis of trimester, two groups were identified. The first-trimester group had 1142 cases (82.7%) and the second-trimester group had 239 cases (17.3%). The placenta was located anteriorly in 582 (42.1%) cases, posteriorly in 664 (48.1%) and fundally in 135 (9.8%). CVS was successful in 100% of the cases but 14 (1%) had inadequate or just maternal sample and returned for additional sampling. Thus CVS was successful in 99% of the cases in a single session. The maximum numbers of needle passes were three (single 73.4%, two 23.7% and three 2.9% patients). However, positive villi were seen in 95.9% following the first pass.

Post-CVS abortion was seen in 20 (1.45%) cases; 17 in first-trimester group (1.5%) and 3 in second-trimester group (1.3%). We had the following minor complications: 8 (0.58%) post-CVS small subchorionic hematoma visible in ultrasound study, 2 (0.15%) severe vasovagal reaction with loss of consciousness for a short period, 8 (0.58%) severe abdominal pain which could be regarded as local peritoneal reaction, 25 (1.81%) experienced post-CVS bleeding or spotting without ending in abortion, 7 (0.5%) post-CVS amniotic fluid leakage and three (0.21%) of them with severe oligohydraminous in the follow-up and finally two abortions selectively on parents' request, while others gave birth to a healthy infant. The complications are shown in Figure 1. Hospitalization was necessary for 2

Table 1—Patient distribution by gestational age. Almost 90% of the patients are within 10–13 weeks range. Mean gestational age was 11.4 weeks. Fetal loss rate in each group has also been shown

Gestational age	No.	Percent	Fetal loss	Percent
9	45	3.3	1	2.22
10	289	20.9	4	1.38
11	508	36.8	7	1.38
12	300	21.7	5	1.67
13	138	10.0	2	1.45
14	51	3.7	1	1.96
15	29	2.1	0	0.00
16	12	0.9	0	0.00
>17	9	0.7	0	0.00
Total	1381	100	20	1.44

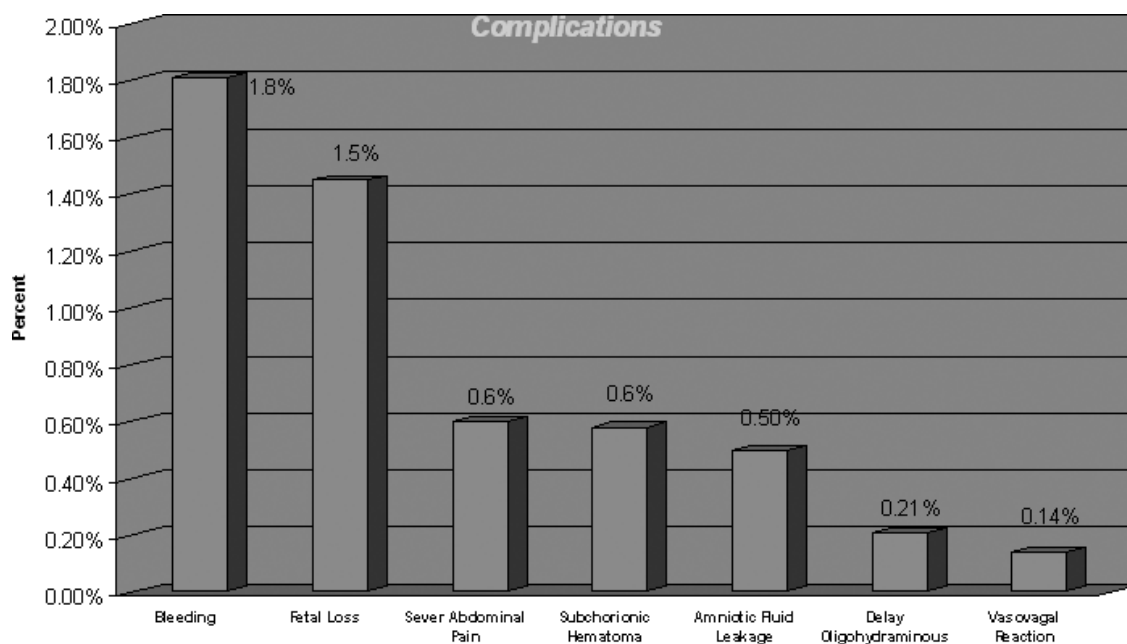


Figure 1—Complication (including early and late) in order of incidence in percent are shown in this histogram. Bleeding or spotting is the commonest complication (1.81%). Fetal loss (1.5%) is below the spontaneous fetal loss rate and delayed oligohydramnios (0.21%) could be a serious problem for the fetus as well

Table 2—Distribution of the patients among the eight country regions showed that most of the patients belong to the Northern region (26.8%). The highest incidence of thalassemia in the country is also in the North. Southwest is the second region which also shows a high percent in our study. Central region is third. Region numbers 1, 2, 6 comprise 69% of the patients. Regions 7, 8, 4, 5 and 2 follow next

Region no.	Name of the region	Country province divisions	Patient distribution (%)
1	Central	Tehran, Markazi, Semnan, Qom, Esfahan, Yazd	20
2	North	Mazandaran, Gilan, Golestan	27
3	Northeast	Khorasan	2
4	Southeast	Sistan va Baloochestan, Kerman	6
5	South	Booshehr, Hormozgan	4
6	Southwest	Fars, Khozestan, Charmahal va Bakhtiari, Kohkiluyeh va Boyerahmad	22
7	West	Kermansha, Koredestan, Hamedan, Lorestan, Ilam	12
8	Northwest	Azarbayejan e Gharbi, Azarbayejan e Sharghi, Ardebil, Zanjan, Qazvin	7

(0.14%) cases. One of the cases had severe post-CVS bleeding that was resolved within 48 h and continued spotting up to 5 days while the other case had active post-CVS amniotic fluid leakage.

By taking into consideration the different regions of the country, the frequencies of the patients were North (26.8%), Southwest (22.4%), Central (19.5%), West (12.2%), Northeast (1.8%), Southeast (5.7%), South (4.3%) and Northeast (1.8%) (Table 2).

DISCUSSION

CVS rapidly became a primary tool for the diagnosis of fetal cytogenetic, molecular and biochemical disorders.

On the other hand CVS were developed to avoid the medical and psychological complications of later prenatal diagnosis by amniocentesis (Wapner, 1997).

Advances in fetal sampling and in detecting mutant globin genes have provided a safe and accurate methodology required for prenatal diagnosis of thalassemia as well. Now we are in a position to discuss the results.

Safety, success and complications

There is no more doubt about the safety of CVS and it is generally accepted.

CVS in the first trimester has become a standard practice (Embury, 1995). In the first three years of starting the CVS in our center we had performed sampling

after 9 weeks according to WHO/EURO published data (Kuliev *et al.*, 1996) and after that we increased our lower level limit to 10 weeks. Even now about 2% of our patients have been sampled after 9 weeks of gestation (Akhlaghpoor and Hosseinipoor, 2005) as the patients who had to travel long distances were not in a position to bear additional costs for longer stay or travel. Establishing more CVS centers in different regions of the country will reduce and eliminate this problem in future.

CVS was successful in 99% of our patients in a single session.

Our second (23.7%) and third needle passes (2.9%) were mainly for taking more villi (Akhlaghpoor and Hosseinipoor, 2005). If we consider the positive result from the first needle pass (presence of villi) the success rate is 96% for single pass. Although using a double needle (co-axial) biopsy technique eliminates a second needle entry into the uterus when insufficient villi is obtained at the first or second attempt, there is no published data to compare the complication of these techniques. Using co-axial technique needs thicker needle diameters; the thickness may compensate the advantage of single uterus puncture. Using single needle technique did not increase the complication rate.

A recently published article showed that the only major complication of CVS is fetal loss (Cederholm *et al.*, 2003). Studies of the additional risk of fetal loss associated with CVS have given divergent results ranging from 0.5 to 4.5% (Alwan *et al.*, 1997).

Other authors have reported a fetal loss rate in CVS cases ranging from 1.65 to 2.4% (1.65% (Scott, 2002), 1.7% (Papp *et al.*, 2002), 1.92% (Brun *et al.*, 2003), 2.3% (Jackson *et al.*, 1992) and 2.4% (Brambati *et al.*, 1988)).

We found an early fetal loss rate of 1.45%. Therefore it can be clearly seen that the CVS fetal loss rate (as a sole major complication) is less than the expected spontaneous fetal loss. However, lack of complete follow-up to 20–28 weeks and parent-induced abortion due to thalassaemic fetus were confounding factors.

But it is also necessary to declare that our patients for thalassaemia screening are usually younger than expected for routine genetical screening in women at risk. So one can expect that we should have lower fetal loss in the young mothers (mean = 26.2 years).

In our series 239 cases were in early second-trimester gestational age (13–17 weeks); by considering this fact our study showed that transabdominal CVS is a useful procedure for prenatal diagnosis of beta-thalassaemia resulting in a lesser fetal loss rate in those couples presenting after the first trimester. Furthermore, we saw lower procedure related fetal loss rate in early second trimester (1.3% vs 1.5%) indicating safer sampling in higher gestational age.

Minor early complications were also reported in different CVS related publications.

These complications include early local peritoneal reaction 0.3% (Brambati *et al.*, 1988), contractions 1.5%, small hematomas 2.1%, and loss of amniotic fluid 0.6% (Saura *et al.*, 1991). Bleeding was the most frequent early complication reported by Hsieh (1986) and

a range reported for spotting from 0.6% (Saura *et al.*, 1991) to 1.8% (Brambati *et al.*, 2002).

Later complications included chorioamnionitis (0.6%) and delayed rupture of membranes and/or oligohydramnios (0.8%) (Hogge *et al.*, 1986). Minor complications were similar to the previously reported complications with some percentage variation (Figure 1).

We had 0.14% vasovagal reaction with a loss of consciousness; one occurred just after the needle insertion and before the sample was extracted. We re-sampled the patient 15 min later. We did not find any rate for this complication in previous studies, nor did we find any hospitalization rate reported. We had 0.14% hospitalization rate. This confirms that the procedure can be done on an outpatient basis.

Implementation of CVS in national control program

To our knowledge prenatal diagnosis of thalassaemia was started in 1991 in Iran. Initially some odd cases were sampled transvaginally by Dr Saremi (gynecologist) in Tehran. Since 1996 regular transabdominal CVS was started and until 1999 almost 350 cases were examined but as the legal abortion issue was not solved the program was not covered by the National Preventive Program.

A national DNA laboratory network including two national genetic reference laboratories and six other laboratories, which had experience with thalassaemia, were initiated in 1999 and began functioning in 2001 (Samavat and Modell, 2004). However, a National CVS network with a reference center has not yet been created. This is one of the drawbacks of the National Control Program.

Each year the number of couples seeking prenatal diagnosis will increase and could exceed 3000 requests for prenatal diagnosis a year as estimated by Samavat and Modell, 2004. The country already has only a few experts in CVS (Samavat and Modell, 2004).

If we consider 200–250 samples per year in each CVS center, 15 CVS centers within a network are expected to meet the demand. The distribution of these CVS centers is also of great importance. Our frequency distribution results demonstrated that 2/3 of the patients were from three regions of the country. (Table 2) This is quite compatible with high thalassaemia prevalence in these three regions. Currently, there is a CVS possibility in the North (Mazandaran), South (Fars), Southeast (Sistan va Baloochestan) and the Northwest (Khorasan) regions, mainly trained by the author. However, the CVS center in the three high prevalence regions should be increased.

It is recommended that the National Program establish a CVS network within its program. The network's role will be to provide expert fetal sampling to patients and additionally provide training to health professionals.

Acceptability of prenatal diagnosis and termination of pregnancy

Fortunately, in recent years in our country the situation has changed. A fatwa was issued in 1998 stating that termination of pregnancy is permissible up to 17 weeks for a genetic abnormality.

This limitation for legal abortion promotes earlier diagnosis, which could be practically more achievable by CVS rather than amniocentesis. Pakistan, a southeast Muslim neighbor country also has a high incidence of beta-thalassemia and its carrier frequency is estimated at 5.4% (Khan and Riazuddin, 1998). In comparison with the Pakistan experience (Ahmed *et al.*, 2000), our report also demonstrates that prenatal diagnosis is feasible and acceptable in a Muslim country.

Rapid evolution of social attitudes toward abortion for a serious fetal abnormality in Iran is also highly relevant to other Islamic countries and for Muslim minorities in high resource countries (Samavat and Modell, 2004).

Our experience during these years has showed that prenatal diagnosis by CVS and subsequent abortion of the affected fetus is acceptable and affordable to most families who are at risk.

The debate regarding termination of pregnancy for thalassaemia is still in progress in several other Muslim countries including Egypt, Tunisia and Maldives; Saudi Arabia as well as Pakistan have concluded that 17 weeks is the appropriate cut-off point (Christianson *et al.*, 2004). Also there are several published data from other Muslim countries such as Turkey (Tuzmen *et al.*, 1996); Malaysia (Chandran *et al.*, 1993) and Lebanon (Zahed and Bou-Dames, 1997) that indicate active prenatal diagnosis of thalassaemia.

Future

Thalassaemia constitutes a major health problem in Iran. Because of the absence of relevant treatment methods to achieve complete cure, and the treatment being expensive, screening the couples-at-risk and prenatal diagnosis with selective termination of an affected fetus is a feasible option to decrease the disease load. The Iranian thalassaemia program is far from complete or perfect (Samavat and Modell, 2004). The prenatal diagnosis program in Iran, started in 1991, can now cover several other genetic disorders. This infrastructure for beta-thalassaemia could also be used for other genetic disorders in future.

The other limitation of fetal diagnosis is that the only avoidance option is abortion. This option is unacceptable to parents who want the child nevertheless.

On the other hand prenatal diagnosis methods will also be improved. There are several research programs in progress for noninvasive diagnosis of the genetic disorders including hemoglobin disorders. Recently published data show more success in identifying the fetal beta (E)-globin gene in maternal plasma (Fucharoen *et al.*, 2003). These results are promising and CVS will be replaced in future by noninvasive methods.

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