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Original Articles

Chorionic Villus Sampling (CVS) for Prenatal Diagnosis of Genetic Disorders in Bangladesh

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Abstract:

Objective(s): *The aim of the study was to identify safety and outcome of trans abdominal Chorionic Villus Sampling (CVS) for prenatal diagnosis of genetic disorders.*

Materials and methods: *This is a retrospective analytical study on women who had undergone trans abdominal CVS. All CVS were done at Fetal medicine centre, Family Foundation, Green Road, Dhaka, from June 2013 to December 2016.*

A total of 286 couples, who were referred for prenatal diagnosis of various genetic disorders were studied. Trans abdominal CVS was done under local anesthesia and real-time ultrasound guidance. A 18G/88mm Spinal Needle (B Braun, Germany) was used. All CVS were performed with the "2 operators" technique. The needle was introduced trans abdominally into the placenta in its longitudinal direction. Once the needle was adequately placed, the chorionic villi were aspirated with a to and fro jiggling movement of the aspiration needle and a suction force was applied through a syringe. Results were recorded and analyzed for descriptive statistics.

Results: *A total of 286 CVSs were performed as outdoor basis. The most common indication was detecting Beta-thalassaemia (82.5%). Other indications were for diagnosis of aneuploidy (9.7%), Hemophilia (3.1%), Spinal muscular atrophy (SMA) (2.4%), Duchenne Muscular Dystrophy (DMD) (2%). Most procedures were done between 11 and 13 weeks (range 11- 14 weeks). Most aspirations (95.1%) were easy; however, in 4.8% cases the aspiration was difficult due to a variety of factors. The overall success rate was 100%. Minor complications like placental hematoma and pervaginal (P/V) bleeding occurred in 2% and 1.3% respectively, which were subsided by conservative management. The procedure related miscarriage within three weeks not occurred in any cases.*

Conclusion: *Trans abdominal CVS under real-time sonography is a useful outdoor procedure for prenatal diagnosis in early pregnancy without significant risk to the mother and the fetus.*

Key words: *Chorionic villus sampling. Prenatal diagnosis*

Introduction:

Chorionic villus sampling (CVS) is the gold standard invasive procedure for first trimester prenatal diagnosis.[1] Trans-abdominal CVS is associated with a lower rate of procedure related miscarriage than trans-cervical CVS. In experienced hands CVS is a safe procedure with overall foetal loss rate of 0.5-1.5%.[2] Lau et al concluded that first trimester trans

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abdominal CVS is an accurate and safe invasive prenatal diagnostic procedure. It should be one of the treatment options available to pregnant women who require prenatal genetic diagnosis.[3] Genetic disorders are a fairly common cause of perinatal morbidity and mortality in Bangladesh. Most of such disorders are either not treatable or the cost of treatment, if available, is out of the reach of the common population. Genetic inherited diseases like thalassemia is a great burden in our society[4,5]. Down syndrome is also very common occurrence. According to WHO reports about 5442 children are born with Down's Syndrome each year in our country [6].

Early prenatal diagnosis and selective termination of the affected pregnancies have become an important component of the management of genetic disorders like Thalassemia and Down's Syndrome. The fetal sample can be obtained by Chorionic Villus Sampling (CVS) through the trans abdominal or the trans-cervical route. The trans abdominal route is considered safer as well as convenient for the patient than the trans cervical route.[2] Other invasive test amniocentesis is also has diagnostic accuracy but it is to be done at later weeks like 15-19 weeks. We started CVS from January 2012 and this study is first of its kind to report in Bangladesh. The objective of this study was to determine the safety and outcome of trans abdominal CVS for the prenatal diagnosis of common genetic/ chromosomal disorders.

Materials and methods:

This retrospective analytical study was done at Fetal medicine centre, Family Foundation, Green Road, Dhaka, from June 2013 to December 2016. A total of 286 couples requested prenatal diagnosis for various genetic disorders were the target population for this study. All were with singleton pregnancies. Before the procedure, couples were counseled about the indications, other options, video demonstration of the procedure, complications of fetal sampling, errors in diagnosis and the termination of pregnancy if needed and its religious implications. A written consent was obtained from all couples.

A preliminary ultrasound scan was done to determine the fetal viability, gestational age, number of fetus, placental location and any other incidental findings that might impact on the procedure. All the procedures were performed via trans abdominal route using a 3.5MHz convex probe. When the gestational age was 11 weeks or more, CVS was carried out immediately. Otherwise, the procedure was deferred till a date corresponding to about 12 weeks gestation. The size and position of placenta was ascertained and a suitable site for introducing the needle on the anterior abdominal wall was selected. The abdominal skin in a radius of about 10 cm was cleaned with 10% povidon iodine solution. Approximately 5-10 ml of 2% xylocain was infiltrated with a 23 gauge spinal needle into the planned whole tract of the CVS needle from the skin to the uterine serosa.

An 18G, 88mm spinal needle (B Braun, Germany) (Figure 1) was first flushed with heparin to prevent clogging. Under continuous ultrasound guidance, the needle was inserted into the abdominal wall, seen traversing the uterine wall and into the maximum bulk of placental tissue (Figure 2). With the needle in place, the chorionic villi were aspirated by to and fro movement.

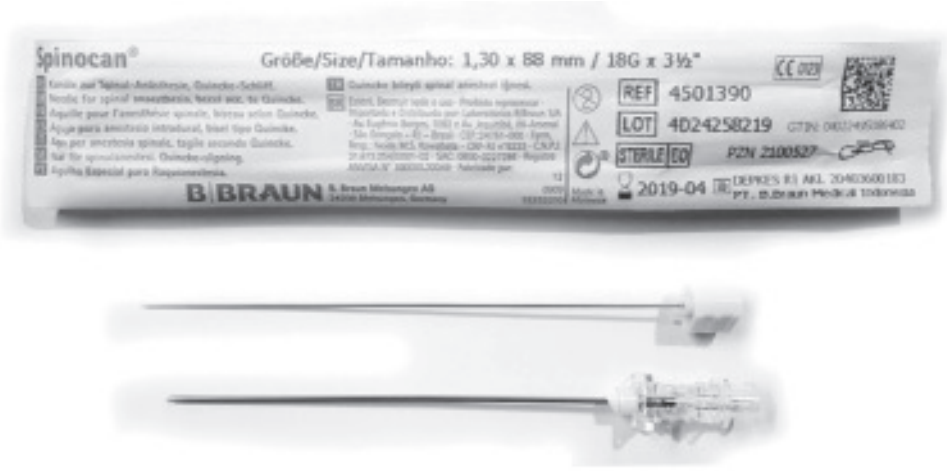

Fig.-1: *Chorionic Villus Sampling Needle. 18G, 88mm Spinal needle (B Braun, Germany).*

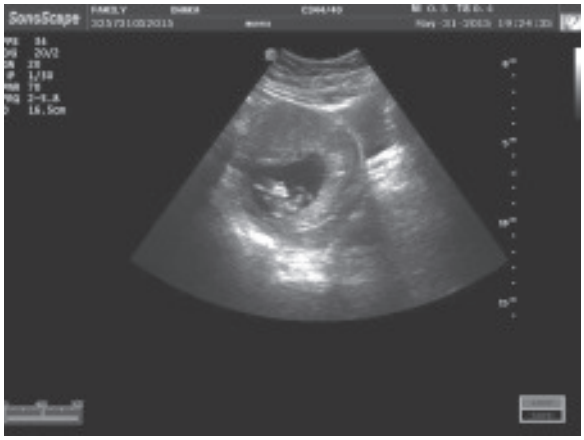

Fig.-2: *Under continuous ultrasound guidance, the needle was seen into the maximum bulk of placental tissue*

A 20 cc syringe containing 5 to 10 ml of normal saline was used for creation of suction force and to aspirate the chorionic tissue. An important step in this procedure was to keep the needle tip visible at all times. The aspiration needle was removed and the retrieved sample of chorionic tissue was placed into petri dishes containing normal saline. In the whole invasive procedure, we adopted the "two operators' technique", (Figure 3) one for manipulating the ultrasound probe to create the image and other for needle maneuvers. The average time duration from insertion of the needle to aspiration of sample was 10 minutes.

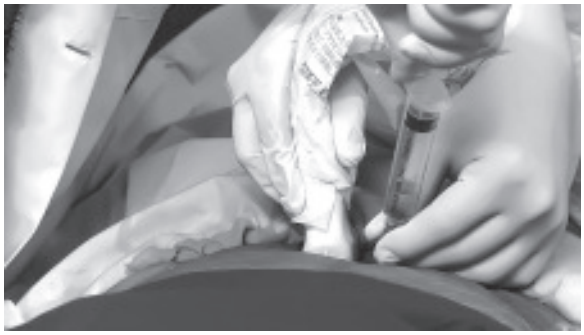

Fig.-3: *"two operators' technique", one for manipulating the ultrasound probe to create the image and other for needle maneuvers.*

Adequate amount (20-25 gm) of grayish white placental villi (Figure 4) confirmed a successful aspiration. Maternal decidua and blood clots were removed and final clean samples were sent to the lab. In case of a poor yield of the sample, a second or rarely a third aspiration attempt was made. The puncture mark was sealed with a sterile water proof bandage. A post-

Fig.-4: *Adequate amount (20-25gm) of grayish white placental villi confirmed a successful aspiration*

aspiration USG scan was done to see the fetal wellbeing, any haematoma formation, or placental separation. The patients were allowed to go home 30 minutes to one hour after the procedure with an advice to take bed rest for 24 hours and avoid journey for 3 days. Prophylactic antibiotics of cephalosporin group and paracetamol tablets were advised for pain relief. Follow-up regarding any complication was done after one week at the time of report collection. Genetic analyses of samples were done in DNA Solution, Dhaka, DNA Lab of Dhaka Shishu Hospital, Genetic Medicine Lab of Sir Ganga Ram Hospital, New Delhi, India. The ethical issues related to prenatal diagnosis, and a possible termination of pregnancy to follow if any, was discussed with the couples. Descriptive statistics were applied to the data using software SPSS-22.

Results:

A total of 286 CVSs were done for various indications during the study period. The mean age of the mother was 30 ± 3.8 years and with a range of 20-40 years. Mean gestational age was 12.5 ± 1.2 weeks. Most procedures (88.1%) were done between 12 and 13 weeks. Regarding placental localization, 210 (73.4%) placenta were anterior, 42 (14.6%) were lateral and 34 (11.8%) were posteriorly placed. The most common indications of CVS were prenatal diagnosis of Beta-thalassaemia/E-Beta thalassemia (82.5%), which included 236 couple. Other indications were for diagnosis of aneuploidy (9.7%), Hemophilia (3.1%), Spinal Muscular Atrophy (SMA) (2.4%) and Duchenne Muscular Dystrophy (DMD) (2%) (Table 1). All the couples referred for prenatal diagnosis by CVS had a history of one or more affected children with their respective indication. Results of the 236 samples for prenatal diagnosis of beta Thalassemia, affected babies

were 54 (22.8%), Carrier 115(48.7) and normal 67(28.3%). In 28 case of chromosome check, only 1 (3.5%) found as a case of Trisomy 21, the other 27 (96.4%) were of normal karyotype. The result of prenatal diagnosis in 9 case of Hemophilia were affected fetuses 2(22.2%), carrier 3(33.3%), normal 4(44.4%). Out of 6 prenatal diagnosis of Duchenne Muscular Dystrophy (DMD), 3(50.0%) were carrier, 2(33.3%) were normal and 1(16.6%) was affected. In case of CVS for Spinal Muscular Atrophy (SMA) disease, out of 7, 2(28.5%) fetuses were normal, 3(42.8%) were carriers and 2(28.5) fetuses were affected (Table 2).

Most aspirations were easy and in most cases (95.1%), adequate sample collection was possible in first attempt, however, in 4.8% the aspiration was difficult due to a variety of factors like fibroids in anterior uterine wall, retroverted uterus and thin placenta. These mothers were called for a repeat CVS one week later, which was successful. The overall success rate was 100% (Table 3).

Minor complications like placental hematoma and P/V bleeding occurred in 2% and 1.3% respectively, which were subsided by conservative management (Table 3). Though abortion rate is 0.5-1% in CVS

Table-I

Maternal Characteristics

Parameters	Mean ± SD	Range
Age (Years)	30± 3.48	20-40
Gestational age (Weeks)	12.5 ± 1.2	11-14
Plaecntal localization	N	%
Anterior	210	73.4
Lateral	42	14.6
Posterior	34	11.8
Indications for testing		
Both parents are carrier for hemoglobin disorder (Beta Trait/Hd E Trait),	236	82.5
Aneuploidy check	28	9.7
Hemophilia	09	3.1
DMD	06	2.0
SMA	07	2.4

Table-II

Results of the Procedure

Indications	Affected		Carrier		Normal	
	N	%	N	%	N	%
Both Parents are carrier of Hemoglobin Disorder.	54	22.8	115	48.7	67	28.3
Aneuploidy Check	1	3.5	00	00	27	96.4
Hemophilia	2	22.2	3	33.3	4	44.4
Duchenne Muscular Dystrophy	1	16.6	3	50.0	2	33.3
Spinal Muscular Atrophy	2	28.5	3	42.8	2	28.5

Table-III

Outcome of Procedure

Parameter	N	%
Successful at first attempt	272	95.1
Repeat Procedure after 1 week	14	4.8
Complications		
Placental hematoma	06	2
Per vaginal bleeding	04	1.3
Miscarriage within 3 weeks	00	00

procedure, we did not have any case of abortion within 3 weeks of the procedure.

Discussion:

Prenatal diagnosis through early fetal sampling has played a pivotal role in the prevention of genetic disorders.[7] Ultrasound guidance adds to the safety for the fetus as well as the mother. Nevertheless, an elaborate learning process to master the technique remains indispensable.[8] Chorionic villus sampling was introduced in the early 80s and since then it has given a new dimension to prenatal diagnosis.[9] Chorionic villus sampling has the great advantage over mid-trimester amniocentesis of producing early results. Moreover, rapid analytic techniques have significantly reduced the waiting time between sampling and diagnosis. Another very important reason for doing CVS is that if it is to be followed by termination of pregnancy then it should be done within a reasonable timeframe defined by consensus.

Positions of placenta were sampled through the trans abdominal route in all cases of our series without much difficulty that makes it the most feasible choice for use in routine practice. This is in contrast to the general opinion that horizontally placed posterior placenta are better sampled with the trans cervical approach [1]. Majority of invasive prenatal diagnostic procedures in the west are performed for individuals deemed to be at high risk for Down's syndrome. Brambati B et al performed CVS on 1,844 women, aged 18–48 years, at 13–20 weeks gestation whose primary indication was chromosomal anomalies and single gene defects in 85% and 15% of cases respectively [10]. In our study majority were done to pick up Thalassaemia Major by CVS to allow timely termination in 1st or early 2nd trimester at a time when complication rate along with maternal tension is decreased due to early voluntary termination of pregnancy [11]. In current study, in contrast to western, only 9.7% cases were done to detect chromosomal anomalies[10]. All of these mothers had previous babies with Down's Syndrome.

Detecting high risk pregnant mother by first trimester screening and referral for definite diagnosis of fetus for aneuploidy by CVS is not yet well practiced in our perspective. According to ACOG, all pregnant women should be aneuploidy screening before 20 weeks, and all pregnant women should be offered diagnostic testing regardless of maternal age or other risk factors [12].

Other indications in our study were Hemophilia, DMD, and SMA, which are similar with study done in other country [13]. Other than Hemoglobinopathies, these inherited genetic diseases are also prevalent in our society due to high consanguineous marriage rate.

Aspirations were easy and in most cases adequate sample collection was possible in first attempt (95.1%). In rest of the cases (4.8%) successful sample collection was done one week later. Aspiration was difficult in these cases due to a variety of factors like fibroids in anterior uterine wall, retroverted uterus and thin placentae. Obesity is also a limiting factor for aspiration. However, we did not find high body mass index as a limiting factor in obtaining specimen. The overall success rate was 100%, which is similar to the study of 144 CVS by Suhaib Ahmed [13], 200 cases by Abeera et al [2] and higher success rate than a large study of 350 CVS by Ajayi et al [14] who reported the success rate of 98%.

Although bleeding and spotting are uncommon, Abeera et al have reported haematoma formation in 1.5% cases and vaginal bleeding in 0.5% cases [2]. We experienced the same minor complications like placental hematoma and P/V bleeding occurred in 2% and 1.3% respectively which were subsided by conservative management.

Pregnancy loss is the most serious complication after CVS. The largest meta-analysis of 29 studies for complications with trans abdominal CVS was performed by Mujezinovic and Alfirevic [15]. The aim of study was to compile a systematic review of complications related to CVS and to provide a benchmark data for counseling and performance and assessment. Pregnancy losses were 0.7%, within 14 days of procedure, 1.3% within 24 weeks of gestation and 2% altogether.

According to Centers for disease control and prevention (CDC), the risk of miscarriage has been attributed to 0.5%-1.0% of CVS procedures and 0.25%-0.50% of amniocentesis procedures [16]. Latest data from randomized controlled trials as well as from systematic reviews and a large national registry study are consistent with a procedure-related miscarriage rate of 0.5-1.0% for amniocentesis as well as for chorionic villus sampling (CVS). Amniocentesis performed prior to 15 weeks had a significantly higher miscarriage rate and also increased the risk of talipes equinovarus than CVS [17, 18]. CVS on the other hand

should not be performed before 10 weeks' gestation due to a possible increase in risk of limb reduction defects [17, 18]. But this was not an issue in our study as all the procedures were carried out between 11-14 weeks.

There was no procedure related miscarriage in any case of this study. We followed up the cases physically or over telephone. Most women had mild to moderate pain following the procedure, which settled with pain killers. The results of this study in terms of miscarriage and other maternal morbidity are comparable to the internationally accepted data. Although the study was done in a single-center, performance may be remarkably good due to very skilled operators, we think this study will help in counseling the prospective parents considering the use of either CVS or amniocentesis and about the benefits, risks, limitations of these procedures.

Conclusion:

Real-time Ultrasound guided trans abdominal CVS is a useful, safe and reliable outdoor procedure for fetal sampling and prenatal diagnosis in early pregnancy and should be considered as a procedure of choice. In experienced hands, the miscarriage rate is very low, thus, it can be safely offered as an alternative to amniocentesis for prenatal diagnosis. It can play an important role in the prevention of genetic disorders and reduce the burden of the diseases that are otherwise incurable.

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