CHORIONIC VILLUS SAMPLING: A SAFE TOOL FOR PRENATAL DIAGNOSIS OF GENETIC DISORDERS

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ABSTRACT

Objectives: To determine the safety and efficacy of chorionic villus sampling (CVS) for early prenatal diagnosis of genetic disorders.

Study Design: Descriptive study.

Place and Duration of Study: Department of Obstetrics and Gynecology, Foundation University Medical College, Rawalpindi, from December 2002 to April 2006.

Patients and Methods: Subjects who had high risk factors like personal or family history of genetic disorders were referred to us for CVS after 10 gestational weeks. Under local anaesthesia and ultrasound guidance (USG) guidance a special chorion biopsy double needle (outer guide and inner aspiration needle) was introduced through anterior abdominal wall into placenta to obtain specimen from chorionic villi. Women were observed for 1-2 hours to notice any immediate complications like uterine cramps and vaginal bleeding. Follow up was done till end of pregnancy to know the outcome. Specimen obtained was sent to laboratory for DNA analysis to diagnose β-thalassaemia in 234 patients and for karyotyping to diagnose Down's syndrome in 17 patients.

Results: Of 252 CVS performed, sample was successfully obtained in 99.6% of cases and only one procedure failed. Most common indication was β-thalassaemia (93.0%). The immediate complications were uterine cramps in 41 (16.3%), vaginal bleeding in one (0.8%) and amniotic cavity puncture in six (2.4%) subjects. Noteworthy was the fact that miscarriage rate was quite low (2.4%). At follow up no evidence of incorrect sampling was reported .Of 234 specimens obtained for DNA analysis, 23.5% were homozygous for β-thalassaemia, to whom termination of pregnancy (TOP) was offered to prevent birth of an abnormal baby.

Conclusion: CVS is a safe and effective method for early prenatal diagnosis of genetic disorders. Its use can help in early detection and prevention of birth of babies with lethal genetic disorders.

Keywords: Genetic diagnosis, Chorionic Villus Sampling, Prenatal diagnosis.

INTRODUCTION

Early prenatal diagnosis by chorionic villus sampling (CVS) initially was proposed as an alternative to routine amniocentesis in the 1970's. CVS is now well established as an invasive test for prenatal diagnosis of genetic disorders like Thalassaemia, Down's syndrome, Hemophilia, Douchenne Muscular Dystrophy, and Congenital Adrenal Hyperplasia. National collaborative trials and large observational studies have reported on the efficacy and safety of CVS: sampling success, karyotyping reliability and fetal loss rates were comparable to midtrimester amniocentesis. [1-3]

CVS usually is performed between 10-12 weeks of gestation, a time when termination of pregnancy (TOP) is technically easier and also risks to the mother with a second trimester termination are more as compared to first trimester [4]. Level of anxiety and discomfort is much less in CVS patients than amniocentesis patients [5]. After amniocentesis it may take 2-3weeks to culture a sufficient number of cells to initiate metabolic or DNA analysis because the amniotic fluid cells are not actively dividing. By the time the results are received by the family, fetal movements may be felt and the mother may be feeling difficulty in making a decision about pregnancy termination.

CVS allows sufficient samples to be obtained for rapid cell cultures and DNA analysis [6, 7]. The sampling is ultrasound guided via transabdominal (TA) or transcervical (TC) approach but the TA-CVS

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has been accepted as the method of choice [8]. Chances of failure are more and multiple insertions may be required with TC-CVS [9]. Sample can be analyzed for determining sex, karyotyping, biochemical and DNA analysis.

The purpose of this study was to determine the safety and efficacy of first trimester chorionic villus sampling for early detection of genetic disorders.

PATIENTS AND METHODS

This clinical study was performed from December 2002 to April 2006 in the department of Obstetrics and Gynaecology, Fauji Foundation Hospital, Rawalpindi. Subjects who had high risk factors like personal or family history or a sibling with a genetic disorder were referred to us for CVS and were included in the study.

The procedure was done between 10-14 weeks of gestation. Initial assessment of the pregnant woman i.e. history and clinical examination were obtained on the first visit followed by a detailed USG to assess number and viability of fetuses, crown rump length (CRL) measurement to know the exact gestation, position of placenta, amount of liquor and presence of fibroids. All these findings were documented on a proforma. An informed written consent was obtained after counseling the couple about the procedure, its indication, complications and follow up. The woman was called at a later date for the procedure in outpatient department (OPD). Prophylactic antibiotic (an intravenous single dose of third generation cephalosporins) was given before starting the procedure. Under ultrasound guidance most suitable site on abdominal wall was chosen and cleaned with pyodine solution followed by infiltration with local anaesthesia (5-10ml of 2% xylocaine). A special chorion biopsy double needle was used to obtain sample. The outer needle (20G) was introduced through the abdomen into the uterine wall with the right hand while holding the USG probe in the left hand to visualize the needle tip. As soon as the needle entered the placenta loss of resistance was felt. The stillet was then removed and inner needle (18 G) was introduced through the outer needle. A 20ml disposable syringe containing 1ml sterile normal saline was attached to inner needle and its plunger pulled half way back to create suction. The sample was sucked in by fine to and fro movements of inner needle and syringe. The inner needle then removed and villi flushed into a sterile petri dish containing normal saline. The outer needle was left in place because in case of inadequate specimen a second or a third attempt could be made. Once sufficient sample obtained outer needle also removed and the puncture site sealed with bandage. Specimen sent to Armed Forces Institute of Pathology (AFIP) for DNA analysis for β-thalassaemia and chromosome analysis i.e. karyotyping for Down's Syndrome. Rhesus negative women were given anti-D prophylaxis after the procedure.

Women were kept under observation for 1-2 hours for any immediate complications like uterine cramps and vaginal bleeding. Follow up was done till the end of their pregnancy to know the outcome and late complications which included miscarriage, intrauterine death (IUD), chorioamnionitis and limb reduction defects in fetuses. Data had been analyzed using SPSS version 10. Descriptive statistics were used to describe the data.

RESULTS

A total of 252 subjects underwent CVS. Majority 184 (77%) of them was less than 30 years. We observed that almost 188 (75.0%) of the couples in our study had consanguinous marriages including first degree and distant relatives. On USG 250 (99.2%) subjects had singleton pregnancy; only two of them had twin fetuses. Sampling was successful in both twin pregnancies via single entry into the placenta using two separate sets of needles. USG findings also showed that (119) 47.0% of the cases had posteriorly located placenta that caused difficulty in obtaining the specimen during CVS while in 133 (53%) anterior placenta was found and procedure was relatively easier in them. Another reason for difficult entry into the placenta was thick abdominal wall in seven (2.8%) cases

Single insertion in placenta was successful in 194 (77.0%) of the women but in 49 (19.4%), double insertion and in nine (3.6%) three insertions were made. Two or three insertions were made in those who had either thick abdominal wall or posteriorly located placenta or both. In three out of nine women procedure had to be abandoned after three insertions and was repeated after one week. Two of them were successful and one still failed.

In this study CVS was performed for two indications: prenatal diagnosis of thalassaemia the main indication in 235 (93.0%) and Down's syndrome 17 (7.0%). Specimen was successfully obtained in all but one (0.4%) procedure failed due to very thick abdominal wall and posteriorly located placenta. So overall in 251 (99.6%) of the cases procedure was successful that was also confirmed at follow up because there was no evidence of incorrect sampling.

On DNA analysis for prenatal diagnosis of thalassaemia, 55 (23.5%) had homozygous fetuses and TOP was offered to them, 122 (52%) were heterozygous, out of them two ended up in miscarriage and one patient had an intrauterine death IUD during eight month of gestation. Karyotyping results showed that one fetus had trisomy 21 but that couple refused TOP (Table-1).

Most common immediate complication that we noticed was uterine cramps in 41(16.3%) of cases. Although in 2.3% of women amniotic cavity was punctured but no case of chorioamnionitis was seen. Miscarriage rate was also quite low (2.4%) (Table-2).

Table-1: Results of DNA Analysis (n=234) and Karyotyping (n=17)

	No	Percentage (%)
DNA Analysis for *β*-Thalassaemia		
Heterozygous	122	52
Homozygous	55	23.4
Normal	57	24.3
Karyotyping		
Normal	16	94
Trisomy21	01	6

Table-2: Frequency of complications (n=251)

	No.	Percentage (%)
Immediate		
Uterine Cramps	41	16.3
Vaginal Bleedin	01	0.4
Amniotic Cavity Puncture	06	2.4
Late		
Miscarriage	06	2.4
Intrauterine Death	01	0.4
Chorioamnionitis	0	0

DISCUSSION

Wapner has documented a high efficiency and reliability of genetic diagnosis by first trimester CVS in 1993 [10]. The high success rate in obtaining tissue (99.2%) was also confirmed by Pergament et al [11]. In our study success rate was 99.6% that compares favorably with other centers [1, 7].

In our study CVS was performed mainly for prenatal diagnosis of thalassaemia 235 (93.25%) because it is more prevalent in our part of the world [12], secondly majority 188 (75%) of the subjects who were referred to us had consanguinous marriages. Data from literature shows that couples from extended families living in endemic areas, and in which consanguinous marriages are common, may be at highest risk [13]. Thirdly they already had siblings with this genetic disorder. This doesn't correlate with international trials where the major indications were advanced maternal age and Down's syndrome [14-16]. In contrast in our study, majority of women 195 (77.4%) was less than 30 years. The reason being lack of medical awareness in our community not knowing the significance of advanced maternal age.

CVS is well accepted as safe and reliable method for prenatal diagnosis in twin pregnancy [17]. Mainly we did CVS for singleton pregnancies but two patients had twin pregnancies. . In both cases twins were heterozygous for β-thalassaemia and women delivered healthy babies without any complications. The sample was successfully obtained in both cases via single entry into the placenta.

Regarding the complications of the procedure, 41 (16.3%) women had uterine

cramps that were relieved by analgesic (mefenamic acid 500mg stat), vaginal bleeding was noticed in one patient (0.4%) and amniotic cavity was punctured in (six) 2.4% of patients, a complication that can lead to chorioamnionitis but fortunately none of our pregnant women developed chorioamnionitis. Vaginal bleeding after CVS is reported in less than 06% of cases and is more common after TC than TA approach [18,19]. Rare cases of infectious complications like chorioamnionitis (0-0.5%) have been reported [20,21]. Moreover TC-CVS carries a higher risk of infection than TA-CVS [22,23]. The miscarriage rate varied in different studies between 1-6% [2,18]. Spontaneous miscarriage frequently occurs during first trimester of pregnancy; the time when CVS is performed so it is difficult to determine whether the loss resulted from the procedure or if it would have occurred spontaneously. Both the American and Canadian collaborative studies on the safety of CVS indicated a post-procedural loss rate lower than had been anticipated [3,24]. In our study miscarriage rate with CVS was 2.4% comparing favorably with international trials [18]. In one trial most of the excess spontaneous fetal losses following CVS occurred in the subgroup of women who underwent TC rather than the TA approach [2]. In three other studies the comparative loss rates for TA and TC CVS, respectively were 2.6 and 2.7% [2] 3.2 and 3.7% [19] and 2.3 and 2.5%[25]. In addition, introduction of the sampling device three times or more is associated with increase in fetal loss rate [12, 20]. In our study TA approach was used and three times introduction was needed in only nine (3.5%) of the cases, which may the contributing factors in reducing the fetal loss rate. In two local studies the fetal loss rates were 0.7 % and 4.1% respectively [26, 27].

At follow up no evidence of incorrect sampling was reported. Subjects, who had either normal or heterozygous fetuses, their pregnancy ended up in delivery of a healthy baby except for the two women who miscarried and one IUD at eighth month of gestation.

CVS has emerged as a safe and efficacious tool for identification of a number of genetic abnormalities. Currently CVS is performed after 10 gestational weeks in order to avoid limb reduction defects, a known complication seen among infants exposed to CVS inutero before 10 weeks [28-30]. Since in our study we performed CVS after 10 weeks of gestation so we didn't notice this complication.

Use of CVS can help in early detection and prevention of birth of babies with lethal genetic disorders [31]. The procedure needs special skills to avoid complications. Performing the procedure after 10 weeks and using transabdominal approach can also reduce complications. There is a great need to create medical awareness in our society by improving community education programs, as well as, opportunities for genetic and prenatal counseling.

CONCLUSION

CVS appears to be a safe and effective method for early prenatal diagnosis of genetic disorders. First trimester prenatal diagnosis has the benefit of producing less emotional stress and making the procedure safer.

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