A Multi-centre Experience of Trans-abdominal Chorionic Villus Sampling in Pakistan

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ABSTRACT

Objective: To determine the safety and outcomes of trans-abdominal chorionic villus sampling technique.
Study Design: Observational study.
Place and Duration of Study: Departments of Obstetrics and Gynaecology, PNS Shifa Karachi, Pak-Emirates Military Hospital Rawalpindi and CMH Lahore, from 2005-2020.
Methodology: A total of 1530 consecutive chorionic villus samplings (CVS) were performed on pregnant females between 10-20 weeks of gestation using the transabdominal approach. Patients were subjected to integrated, stepwise sequential screening. Analysis of data was based on demographic features, indications for sampling, gestational age, attempts of CVS, needle aspiration time, assessment, placental location, sample yield, complications, pain estimation by visual analogue scale (VAS), CVS culture results and pregnancy outcomes.
Results: The most common indication for CVS was couple having thalassemia traits and history of having a thalassemia major child previously (55.2%). Pain was the most common complication (64.1%). Procedure-related pregnancy loss (considered to be till 20 weeks of gestation) was observed in two cases (0.1%) only. The most common abnormal karyotype was found to be β-Thalassemia trait (23.6%) followed by β-Thalassemia major (22.1%) and Trisomy 21(16.8%). No abnormality was detected in 33.5% of the cases. Five hundred and eighty-nine (38.4%) interruptions of pregnancies were done on the basis of CVS results.
Conclusion: CVS is a safe and useful technique for sampling in prenatal diagnosis of genetic disorders, markedly affecting the management.

Key Words: *Chorionic villus sampling, Pre-natal diagnosis, Karyotype.*

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INTRODUCTION

Aneuploidies and hereditary disorders are significant causes of perinatal, neonatal, infant, and childhood morbidity and mortality. Worldwide 240 000 newborns die worldwide within 28 days of birth every year due to congenital anomalies.[1] These disorders may cause long-term debility, which can cause substantial effect on individuals, their families, overall healthcare systems and society as a whole. These anomalies are more prevalent in developing countries like Pakistan predominantly because of high prevalence of consanguineous marriages and high birth rates.[2]Chorionic villus sampling (CVS) is an invasive diagnostic procedure that is relatively safe if done in the prenatal period before 14th week of gestation. It has been demonstrated to be helpful in diagnosing pregnancies that are at risk of poor outcomes.[3]

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CVS is considered superior to amniocentesis as a method of prenatal diagnosis due to the gender amount of DNA extracted.[4] Furthermore, it has been observed that Spontaneous miscarriages occur more often after early amniocentesis as compared with transabdominal CVS.[5]

The spectrum of congenital disorders that can be diagnosed prenatally and evaluated the possible outcomes of pregnancies diagnosed with congenital disorders at tertiary care hospitals of three major cities in Pakistan. These evidence-based results will be conducive in counselling of the patients and timely management of high-risk populations in future. The objective of this study was to determine the safety and outcomes of the trans-abdominal chorionic villus sampling technique.

METHODOLOGY

This study was conducted at the departments of Obstetrics and Gynecology, PNS Shifa, PEMH Rawalpindi and CMH Lahore. All pregnant females, who were referred for CVS, were included in the study. Patients were subjected to integrated, stepwise sequential screening before being marked as candidate for invasive diagnostic testing. This stepwise scrutiny for CVS included obstetric history, history of chromosomal or genetic disorders in the family, parents known carriers of genetic disorders,

abnormal first-trimester screening results including ultrasound and biochemical markers, recurrent fetal losses, and advanced maternal age. The exclusion criteria were multiple pregnancies, threatened miscarriage, patients with known comorbidities such as preexisting hypertension, diabetes mellitus, anti-phospholipid syndrome (APS), thyroid disease, renal disease, systemic lupus erythematosus (SLE) or any other medical illness. These patients were booked at or before 10 weeks of gestation or were referred from peripheral hospitals specifically for CVS. A booking scan was done in all patients to confirm the gestational age. CVS was planned between 10 to 13+6 weeks of gestation. Demographic data were collected using a questionnaire like age, occupation, ethnicity, and consanguineous or non-consanguineous marriage. The couples were counselled about the risks and limitations of CVS and whether they would consent for interruption of pregnancy if indicated by CVS report. Trans-abdominal CVS was planned after taking written informed consent from the couple, confirming the gestational age and coagulation status as out-patient procedure.

All CVS procedures were performed trans-abdominally. Fetal viability and placental location were confirmed by ultrasonography. CVS sampling was done with a 20 G Chorionic villus biopsy needle (Rocket, United Kingdom) attached to a syringe using continuous negative pressure aspiration technique. All CVS procedures were conducted under continuous ultrasound guidance and strict aseptic measures. Skin was disinfected with povidone solution and local anaesthesia was given by injecting 1% lignocaine. Under direct vision with ultrasound guidance, the needle was inserted through the uterus and into the placental tissue. After reaching well into the placenta, the stilette was withdrawn and tip of the cannula was moved 5 -8 times within the substance of the placenta with simultaneous application of negative pressure created by manual aspiration in a 10 ml syringe fixed to the cannula. In case the yield of the sample was inadequate, the cannula was re-inserted, repeating the same procedure. At the end of procedure, needle was removed followed by application of pressure dressing. Patient was demonstrated fetal cardiac activity on scan for reassurance. Rh Anti-D prophylaxis was given when indicated. Patient was kept under observation for 30 min following the procedure. The samples were then sent for cytogenetic studies and patients were called for follow-up with report after a period of 14 days.

The data was compiled and statistically analysed by SPSS 26. Descriptive analytical tests were applied to calculate the frequencies, means, and standard deviations for demographic data, indications, attempts, aspiration time, assessment, placental location, sample yield, complications, pain on VAS, follow-up, CVS report and pregnancy outcome. Statistical test (ANOVA) was applied to see the relation of complications with aspiration time, number of attempts, placental location, assessment *etc*. This study was carried out after seeking approval by the respective hospital ethical review boards.

RESULTS

A total of 1,530 patients underwent CVS procedure with mean maternal age of 30.5±5.3 years. Most of the women were consanguineously married (72.9%), housewives (90.4%), and belonged to the province of Punjab (62%). Three hundred and ninety-six (25.9%) were Pashtuns, Balochi 30(2.0%), Sindhi 40(2.6%), and 115(7.5%) were Kashmiri. The CVS procedures were performed mostly on multigravidas (70.1%), between gestational age of 10-14 weeks n = 1199 (78.4%). The most common indication for which CVS was performed was couple having thalassemia traits and history of having a thalassemia major child previously (55.2%, Table I).

Table I: Indications of CVS.

Indications	n (%)
Abnormal 1st-trimester ultrasound screening	31(2%)
Previous Sickle cell disease child	10(0.7%)
Family history of chromosomal/genetic disorder	78(5.1%)
Parents known carriers of genetic disorder	95(6.2%)
Advanced maternal age	96(6.3%)
Previous Down's syndrome child	332(21.7%)
Previous thalassemia major child/ couple thalassemia minor	845(55.2%)
Previous Duchene child	16(1%)
Previous cystic fibrosis child	5(0.3%)
Recurrent fetal loss to rule out aneuploidies	22(1.4%)

Table II: Aspiration time, placental location, sample yield.

Variables		n (%)
Aspiration time	<30 sec	905 (59.2%)
	30-60 sec	595 (38.9%)
	1-2 min	23 (1.5%)
	>2min	7 (0.5%)
Placental location	Anterior	678 (44.3%)
	Anterior low-lying	292 (19.1%)
	Posterior	394 (25.8%)
	Posterior low lying	166 (10.8%)
Sample yield	Plentiful (numerous villi)	819 (53.5%)
	Adequate (6 clear villi)	711 (46.5%)
Pain On VAS	VAS-1	197 (12.9%)
	VAS-2	838 (54.8%)
	VAS-3	387 (25.3%)
	VAS-4	76 (5%)
	VAS-5	16 (1%)
	VAS-6	16 (1%)

Majority of the CVS were carried out in the first attempt (84.4%) with easy access (50.3%) having anterior placental location (44.3%) with maximum of CVS procedures taking an aspiration time of <30 seconds (59.2%) with plentiful sample yield (53.5%) (Table II).

Pain was the most common complication observed (64.1%) which was analysed by Visual Analogue Score (VAS) which showed mild pain (VAS-2=54.8%) in most of the patients (Table II). Procedure-related pregnancy losses were considered in till 20 weeks of gestation which were observed in two cases (0.1%). No increased pregnancy loss rate was observed following 2nd attempt. The most common abnormal karyotype was found to be β-thalassemia trait (23.6%) followed by β-thalassemia major (22.1%) and trisomy 21(16.8%). No abnormality was detected in 33.5% of the cases. Five hundred and eighty-nine (38.4%) interruption of pregnancies were done on the basis of final diagnosis from CVS (Table III).

The occurrence of complications was significantly related to aspiration time (p=0.009) and placental location (p=0.019), access to villous tissue (p=0.001), pain during procedure (p=0.039) and pregnancy outcome (0.017) while the complications were not significantly related to maternal age (p=0.87).

Table III: CVS-based diagnoses and pregnancy outcomes.

		n (%)
CVS samples	No abnormality detected	513 (33.5%)
	Trisomy 21	257 (16.8%)
	Trisomy 18	3 (0.2%)
	Beta thalassemia major	338 (22.1%)
	Beta thalassemia trait	361 (23.6%)
	Duchene muscular dystrophy	4 (0.3%)
	Lost to follow	46 (3%)
	Cystic fibrosis	8 (0.5%)
Pregnancy outcome	Miscarriage (procedure-related loss upto 20 weeks)	2 (0.1%)
	Pre-term delivery	32 (2.1%)
	Term delivery with fetal abnormality	38 (2.4%)
	Term delivery	749 (49%)
	Interruption of pregnancy	589 (38.4%)
	Lost to follow-up	120 (7.8%)

DISCUSSION

CVS is considered a relatively safe procedure for prenatal diagnosis worldwide with much lower procedure-related risks of miscarriage.[6,7] It is being performed for prenatal diagnosis of various indications. Hereditary disorders were the most common CVS indications.[8] In this study, CVS was performed for indications such as advanced maternal age previous Sickle Cell disease child, previous Down's syndrome child, previous thalassemia major child or couple with thalassemia minor, previous Duchene atrophy child, previous Cystic fibrosis child, parents known carriers of any genetic disorder, history of recurrent fetal loss to rule out aneuploidies. Of the 1530 CVS performed, 513(33.5%) were normal, 971(63.4%) had some form of abnormality, whereas 46(3%) cases were lost to follow. These statistics are in disparity to the work done by Ozturk *et al.* in which 385(69.7%) out of total 552 CVS were normal and 141(25.5%) were abnormal. The indications mostly included atypical screening results, cystic hygroma/oedema and increased nuchal translucency.[9]

The most frequent indication for which CVS was performed was couple having thalassemia trait and history of having a thalassemia major child previously (55.2%) which is comparable with a recent study done in Bangladesh which showed thalassemia to be the most common congenital monogenic disorder.[10] This finding can predominantly be ascribed to the high prevalence of consanguinity in the population which was found to be 34% in this study. In Pakistan, babies with SMN gene deletions were reported to be born out of consanguineous marriages in 68% of cases.[8] This may also be attributed to the availability of diagnostic facilities for thalassemia major/minor in the form of haemoglobin electrophoresis which has led to more and more couples opting for invasive testing for this condition in their offspring. Prenatal diagnosis is only being offered at selected centres and for selective cases in Pakistan and as a result many cases remain undiagnosed and unreported. The actual statistics may be much higher. Since it was a multi-centre study, patients from all over Pakistan were included and 62% were from Punjab which is consistent with another study done on Thalassemia at the Armed Forces Institute of Pathology.[11] A study conducted at Bahawalpur showed Saraikis (81.2%) to be the most prevalent ethnic group which is a geographic location effect.[12]

Majority of the CVS were carried out in the first attempt (84.4%) with easy access (50.3%) having anterior placental location (44.3%) with maximum of CVS procedures taking an aspiration time of <30 seconds (59.2%) with plentiful sample yield (53.5%). Pain during the procedure was the most common complication observed (64.1%) and is consistent with another study conducted.[13] The authors analysed pain by VAS which showed mild pain (VAS-2=54.8%) in most of the patients. Although the pain was effectively controlled by local anaesthesia, some other complementary pain relief could also be used to make this procedure and experience pain-free for the patient. In a study conducted in USA, it was found that during transabdominal CVS procedure, the use of 1% lidocaine subdermal injection resulted in less pain as compared to topical ethyl chloride anaesthetic spray.[14]

Procedure-related pregnancy losses were considered to be till 20 weeks of gestation which were observed to be 2 cases (0.1%) which are lower than the results obtained by Gil *et al.* in which the miscarriage incidence observed in the CVS group was 2.1%.[15] Risk of fetal loss after CVS observed in another study was about 3.5% above the patient's background risk.[16]

The most common abnormal karyotype was found to be β-Thalassemia Trait (23.6%) followed by β-thalassemia major (22.1%) and trisomy 21(16.8%) which is consistent with the study done in China.[17] In another study conducted in Rajasthan state, 952 individuals were screened for β-thalassemia and of them 69 (7.25%) were found to be β-thalassemia positive.[18] No abnormality was detected in 33.5% of the cases. Five hundred and eighty-nine (38.4%) interruption of pregnancies were done on the basis of CVS reports. All interruption of pregnancies were uneventful with no major complications observed. The rate of Preterm births (2.1%) did not exceed normal rates observed in the general population.[19] Seventy-two (4.70%) complications including miscarriages, pre-term deliveries and term deliveries with fetal abnormalities were seen which is in accordance to another study which shows pregnancy loss rates after CVS to be comparable to losses in pregnancies without this procedure.[20]

The limitation of this study was the rate of lost to follow-up cases which was 7.8% overall. This can be elucidated by the fact that for prenatal diagnosis, referrals of patients were mainly done from the periphery. With dedicated staff for counselling and following the patients, this rate can be significantly brought down and patients can be benefitted with better pregnancy outcomes.

CONCLUSION

CVS technique for prenatal diagnosis is a very effective, safe, and useful tool if done at the appropriate time recommended in early

2nd trimester of pregnancy. As its results markedly affect the pregnancy outcomes, it should be generously offered to all couples with indication for prenatal diagnosis for early detection, effective control, and management of congenital disorders in Pakistan.

ETHICAL APPROVAL:
Ethical approvals were obtained prior to the initiation of the research work.

PATIENTS' CONSENT:
Informed consent was obtained from each patient included in the study and consent was taken to publish the data concerning their case.

COMPETING INTEREST:
There is no competing interest to declare by any author.

AUTHORS' CONTRIBUTION:
SB: Conception and design of work. The acquisition, analysis and interpretation of data. Drafting the work and reviewing it critically for important intellectual content. Revision of the manuscript.
RI: Writing manuscript, data analysis and interpretation of data, and revision of the manuscript.
All the authors have approved the final version of the manuscript to be published.

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