CHORION VILLUS SAMPLING

(A Preliminary study with improved transcervical technique and cell culture)

By

V. R. AMBIYE, M. V. AMBIYE, SAKSHAT MHATRE, A. M. SHANBHAG, P. R. VAIDYA AND P. S. PATKI

SUMMARY

A preliminary experience of fifty transcervical chorion villus sampling under ultrasound sector scan and its culture for prenatal diagnosis of genetic disorders is presented.

An innovative technique of using 4 M.M. menstrual regulation canula and syringe, which can obtain chorionic tissue sufficient for both culture and chromatin study is described. The culture technology which can give reports within 12 hours is outlined.

The pitfalls, difficulties, complications and the scope of chorion villus sampling in prenatal genetic diagnosis is discussed.

Over the past decade sampling and processing of chrionic villi has been recognized as the best method for prenatal diagnosis of genetic disorders.

The chorionic tissue originates from the zygote and has the same genetic constitution as the foetus. Moreover it can be easily and safely sampled between 8-12 weeks of gestation.

The study was conducted at Department of Obstetrics and Gynaecology at L.T.M.M. College and Sion Hospital, Sion, Bombay 400 022, India in collaboration with Department of Radiology. The culture technology and karyotyping was developed at Department of Anatomy, J.J. Group of Hospitals and Grant Medical College, Bombay 400 008, India.

From: Department of Obstetrics and Gynaecology, L.T.M.M. College and L.T.M.G. Hospital, Sion, Bombay-400 022. And Department of Anatomy, Grant Medical College, Bombay 400 008.

Accepted for publication on 5-4-88.

Technique of C.V.S.

We have developed our own technique of transcervical aspiration of chorionic villi under direct vision by sector scan. We wish to describe it in detail.

All procedures were strictly limited to 8-12 weeks of gestation. The procedure was carried out as an O.P.D. procedure in the radiology department. No anaesthesia or analgesia was used. Inj. Atropine 1 ampule I.M. was given half an hour prior to the operation. Ultrasound scanning of the gestational sac is carried out to confirm the size of the gestation, localisation of the implantation site, chorion frondosum etc.

The perineum and vagina is thoroughly cleaned with Betadine 5% solution. Sims speculem is inserted to retract the

posterior vaginal wall and cervix is steadied with Ellis forceps on the anterior lip.

In the beginning of the study we used to do chorion biopsy using a traditional method of passing a metal chorion biopsy conula through the cervix upto the chorion frondosum. A polythelene canula of No. 16 gauge was then introduced through the metal canula and negative pressure applied with a syringe. The tissue collected was flushed into the media and screened under the dissecting microscope. This method was used for the initial 15 cases of C.V.S. However there were 2 failures and in 3 cases the tissue obtained was insufficient for culture, although sufficient for sex chromatin study. Therefore we decided to do chorion biopsy using menstrual regulation canula (4 mm.) and Karman syringe. The initial procedures were same as before. A menstrual regulation canula was introduced under ultrasound control through the cervix till it reached the chorion frondosum. Suction was created in the Karman syringe after pinching the adoptor. The adoptor was then attached to the canula and the pinch valve on the adoptor was released so that suction was applied. The suction was maintained for one minute without moving the canula to and fro or rotational movement as in menstrual regulation. After 1 minute the pinch valve on the adaptor was pressed again and the canula alongwith the syringe withdrawn. All the contents of the canula were transferred to the bottle containing the culture medium. To the naked eye, the chorionic villi are visible, as pinkish finger like projections floating in the media. The chorionic tissue is further identified under dissecting microscope. After confirming that the tissue obtained is chorionic are not maternal decidual, the sample is sent to the laboratory for chromosomal study and culture. With this method the chorionic tissue obtained is sufficient for both sex chromatin study and chromosomal culture.

The most common difficulty encountered is, directing the canula to chorion frondosum in acutely anteflexed or retroflexed uteri. This was overcome by introducing a maleable stylet in the menstrual regulation canula and angulating it according to the need. The operation table can also be tilted either head up for retroflexion or head low for anteflexion. At the end of the procedure foetal heart was checked on the ultrasound and patient made to lie down for half an hour. Invariably there was slight bleeding from the os at the end of the procedure which stopped within 12 hours. She was advised to take complete rest for one day and was given prophylactic uterine relaxants like isoxuprine, oral progestagens and antibiotics for 5 days. She was requested to attend follow-up after 15 days or earlier in the event of bleedings.

Methodology of sex chromatin study and chorion villus culture

The processing of the sample begins within half an hour of the collection. The tissue obtained was teased under the microscope and the villi were added to 5 ml. of fresh T.C. 199 medium alongwith 0.1 ml. of colchicine. The media kept at 37°C. in the incubator for half hour. This was known as planting of C.V. culture.

After half hour the contents were transferred to a centrifuge tube and allowed to settle. The supernatant fluid removed and 5 ml. of 1% hypotonic solution of sodium citrate added. It was allowed to stand for 10 minutes at 37°C. The procedure was repeated and after removing the supernatant fluid 3 ml. of fixative solution (aceitic acid + methanol) added.

After ½ hour 7% acetic acid was added and the solution mixed well. This procedure was known as harvesting the culture.

A drop was then put on the slide, allowed to dry and stained by Giemsa staining method. A photograph of a good field taken, developed, and printed. The chromosomes were cut and arranged on a karyotype chart.

For sex chromatin study there is no need for planting and harvesting, 7% acetic acid is added to the sample and is mixed well. After preparing the slide it is stained by toludine blue. At least 100 cells are counted and percentage of barr body is calculated

With our method the reports are ready within 12 hours of C.V. sampling.

Results

Over a period of 6 months from January to June 1987, total number of 50 C.V.S. were performed by us at L.T.M.G. Hospital, Bombay 400 022 and were analysed at department of Genetics at J.J. Group of hospitals and Grant Medical College, Bombay 400 008.

Of these first 15 were performed by using metal chorion biopsy canula and ordinary glass syringe. In these cases we could do sex chromatin study in 12 and culture study in 10 only. In 3 cases tissue obtained was decidual while in 2 the tissue although chorionic was insufficient for culture. (Table I).

TABLE I

No. C.V.S.	50
Failure of technique	2
Insufficient Tissue	3
Maternal Contamination	1
*Threatened Abortion	1
Sepsis	Nil
Pregnancy/Labour complications	Nil

(* Patient continuing pregnancy after conservative management).

After this setback we decided to do C.V.S. using M.R. Canula (5 MM.) and M.R. Syringe. By using this method we could do both sex chromatin and culture studies in all 35 consecutive cases. Only one patient had bleeding even after 12 hours of the C.V. sampling. She was admitted to the hospital and was treated as threatened abortion. Twentyeight have already delivered and the sex of the baby tallies with the results obtained on sex chromatin study and culture. Ten cases were lost to follow-up and were not attending the antenatal clinic. The pregnancy and labour were uneventful except threatened abortion in one.

Table II shows distribution of cases according to gestational weeks and method of C.V.S. Failures were in the group of 8-9 weeks and when usual metal chorion

TABLE II

Distribution of cases according to Gestational weeks and method of C.V.S.

Weeks	No.	Method		Failure
		Metal canula	*M. R. Canula*	
8-9	25	15*	10	*2
10-11	20	—	20	—
12-14	5	—	5	—
Total	50	15	35	2

(*Failures were in conventional group using metal canula and syringe).

biopsy canula and syringe was used for sampling. We feel that sampling is more successful in later weeks of pregnancy i.e. after 10 weeks when chorion frondosum is well developed and chorionic tissue is abundant.

Discussion

Chorion Villus sampling (C.V.S.) as an alternative to second trimester amniocentesis has been suggested since late 1960 (Mohr. J., 1968). In 1975 Tietung hospital from China reported blind aspiration of chorionic villi by transcervical route for prenatal sex determination of the foetus. Since then a number of techniques have been reported. (Table III). Of these transcervical techniques are useful in first trimester while transabdominal ones can be used only in later advanced stages of pregnancy. The combined use of both is particularly suitable for obtaining samples from both or more gestational sacs in twin or multiple pregnancies.

TABLE III
Various techniques of C.V.S.

(A) Transcervical Blind Aspirate (Tietung Hospital, China, 1975)
(B) Ultrasound Guided Transcervical Aspirate (Maxwell. D., 1985)
(C) Contact Hysteroscopy (Rodect, C. H., 1983)
(D) Ultrasound Guided Transabdominal Aspirate (Nicolaides, 1986)
(E) Foetoscopic Aspirate (Nicolaides, 1986)

We have performed transcervical C.V.S. using metal chorion biopsy canula (15) and 4 mm. menstrual regulation canula and syringe (35). The procedure was performed under ultrasonographic control uneventfully in most of the cases. The failure rate was 4% with both the cases recorded in the earlier phase of the study by using metal C.V.S. canula and early weeks of pregnancy. Later on, when M.R. Canula and syringe was used, there was no failure and tissue obtained was sufficient for both sex chromatin study and culture. We feel chorion biopsy is more likely to be successful after 10 weeks of gestation when chorionic tissue is abundant.

As regards complications. early complications included light vaginal bleeding (10-15%) a characteristic feature of transcervical sampling, ascending uterine infection (0.1-0.2%) C.V.S. does not appear to affect obstetrical complications in second half of the pregnancy. The rates of placental disorders (1.6%), premature rupture of membranes (0.7%) and premature delivery (6.1%) are well within the expected limits of general population. (Brambati, B., 1987). In our study there was only one case of threatened abortion (2%), otherwise the pregnancy and labour was uneventful.

The diagnostic accuracy of C.V.S. both chromatin study and culture is very high. In our study sex of the foetus tallied with the reports in all the cases. However several problems are envisaged viz. cytogenetic discrepancy between placenta and foetus, effect of decidual contamination or biochemical study and meiotic recombination of D.N.A. analysis by restriction fragment length polymorphisms. (Galjaard, H., 1986).

In conclusion C.V.S. is a safe, reliable and simple alternative to amniocentesis in prenatal diagnosis of genetic disorders. The anxiety and long wait upto second trimester, and psychological and obstetrical complications of second trimester

abortion are minimised by this new diagnostic modality. However it remains an applied research procedure which should be offered to carefully evaluated cases for genetic study and not for diagnosis of foetal sex for selective female foeticide. More extensive study is required to obtain risk figures and better knowledge of potential pitfalls in the diagnostic process.

Acknowledgement

Our sincere thanks are due to Dr. (Mrs.) Lata Mehta, Ex. Head Dept. of Anatomy Grant Medical College and Dr H. C. Shah, Ex. Head Dept. of Radiology. L.T.M. College for their kind permission to do the study.

He also thank Dr. S. V. Nadkarni, Dean, L.T.M.M. College for allowing us to publish the study.