Iranian J. Publ. Health 1992, Vol. 21, Nos. 1-4

CYTOGENETIC PRENATAL DIAGNOSIS ON 66 CHORIONIC VILLUS SAMPLES IN IRAN★

P. MEHDIPOUR[1] , PhD

KeyWords: *Chorionic Villus Sampling (CVS); prenatal Diagnosis; Cytogenetics; Iran.*

ABSTRACT

A total number of 66 chorionic villus samples were cytogenetically investigated. The samples consisted of 30 experimental CVS from spontaneously aborted material and 36 from live gestations.

80% of the samples were successfully grown. of the 30 cases 40% (12) and 33% (10) contained a normal female and a normal male karyotype, respectively, 3.3% (1) and 3.3% (1) had abnormal karyotypes (47,XX,+21; 47, XY, +18), respectively, and 16% (6) of the cultures did not grow.

of the 36 CVS, 80. 5% of the trophoblasts grew. 50% and 30.5% had normal karyotypes with 46, XY and 46, XX chromosome constitutions respectively, of those with a male karyotype, one case was revealed to have two mitoses with 47+XY,+21 karyotype and was considered to be a Mosaic with a minor abnormal clone. One case

---

★ This manuscript was presented at the Annual congress of Iranian pediatrics' Society, Oct. 1991, Tehran, Iran.

1- Dept. of Human Genetics, School of public Health, Tehran University of Medical Sciences, Tehran, Iran. P.O.Box 14155-6446.

(2.7%) was a twin; 5.5% of the samples did not grow and 11.1% of the villi were inadequate.

No serious complications occured after CVS. 12 girls and 9 biys were delivered at term and cytogenetic findings on the CVS were postnatally confirmed for normal cases and even for a Mosaic case.

INTRODUCTION

Several diagnostic tools are available to perinatologist for detecting many of the fetal disorders in utero. Among these are amniocentesis (AC) and chorionic villus sampling (CVS).

The preventive approach of CVS and the desirability of the procedure, as early as possible, at the period of the first trimester, i.e, between 8 and 12th weeks' gestation, are considered very important. It is the purpose of this article to present a first experience in Iran of CVS, cytogenetic diagnosis of the trophoblasts and its importance in genetic counselling.

The cytogenetic indications for CVS included:

1. The materanl age of 33 years or over.
2. The previous birth of a sibling with a chromosomal abnormality.
3. Chromosome aberration in either parent.
4. The mothers who were carriers of X-linked recessive disorders.

Successful CVS has been carried out by several investigators (6,7,14). The method, however was much improved by Simoni (12). By April 1983 only five centers were active in this field and by 1988 the number reached 43 including Iran (9).

MATERIALS AND METHODS

The material used in the present investigation of CVS in Itan were trophoblasts from 66 samples, thirty obtained from trophoblasts of inevitable abortions between 7 and 13 weeks' gestation and thirty six by CVS from live gestations.

The samling of villi (5-20mg) was performed trancervically under ultrasonic guidance (Hitachi EUB) transducer, using a 1/2mm plastic biopsy canula with a nailable metal trocar (portex trophocan).

The patients were placed in a lithotomic position and prepped and drapped for the procedure.

Examination of chromosomes was made in dividing cells of trophoblasts either directly or after a short-term culture, using a culture medium containing 20% new born calf serum and 1% antibiotics (7, 12) . cell division was stopped by adding colchicine (0.1 μg/ml). After treating with a bypotonic solution (o.09% Nacl), fixative (3 parts methanol: 1 part acetic acid) was added and slides were prepared. Giemsa and quinacrine banding techniques were used and mitoses were photographed with a Leitz - Microscope.

RESULTS AND DISCUSSION

The distribution of patients according to their age, gestational age, pertinent history and cytogenetic findings is given in Table 1. The majority of the cases had been referred because of the maternal age.

Tables 2 and 3 demonstrate the cytogenetic results of 30 CVS of aborted material and 36 CVS of live gestations, respectively.

80% (53/66) of the total trophoblast samples and 80% (24/30) of the aborted material were successfully grown. 40% (12/30) and 33. 6% (10/30) were revealed to have normal female and normal male karyotypes, respectively. 3.3% (1/30) and 3.3%

(1/30) had abnormal karyotypes with trisomy 21 and 18 respectively (47, XX, +21 and 47, XY, +18), and 16.6% (6/30) of the cultures did not grow.

From among 36 CVS of the live gestations, with follow-up studies, 80. 5% (29/36)were successfully grown. 50%(18/36) and 30.5%(11/36) revealed to have normal female and male karyotypes, respectively. One (2.7%) was a twin whose CVS' could only be obtained from one of the placentas and the cytogentic finding showed the presence of polyploidy in the majority of the mitoses, 5.5% (2/36) of the trophoplasts did not grow, and ll.1% (4/36) of the samples were inadequate.

Cytogenetic follow-up studies were also made on the amniotic fluid of some the cases at later stage of gestation and on peripheral blood of the delivered cases. Twenty one casses progressed normally, of whom tewlve girls and nine boys were delivered at term; cytogenetic findings on CVS were also confirmed.

The evaluation of decisions concerning the continuation or termination of a pregnancy on the basis of prenatal diagnosis has been reported previously (3). The paradigm of mosaicism in CVS is very sensitive and important.

There are some report available on the diagnosis of true and pseudo - mosaicism, its problem and reliablilty in CVS (2,8,13). However, the mosaicism may occur in only 0.2-0.4% of pregnancies, and pseudomosaicism, due to a trisomic clone, occurs in 2% to 3% of amniotic fluid culture (1,4,10,11), amongst which mosaicism for trisomy 20 with no congenital defect is also reported (5).

The present cytogenetic findings of case No. 23 were shown to contain 46,XY katyotype, as a major clone (N=17,88%), accompanied by two trisomic mitoses, i.e., 47,XY,+,21,at 71½ weeks' gestation. The parents were counselled and recommended to have a routine follow-up stusy in order to determine the precise percentage of mosaicism. Because of the psychological problem of the parents, the pregnancy was continued without performance of any further amniocentesis and fetal blood sampling and delivery occured at term. A baby boy with mild clinical features, characteristic of Mosaic - Down's syndrome, was born and cytogenetically investigated and the true mosaicism was also postnatally confirmed (46, XY/47,XY, + 21: N=43, 86% and N=7,

14%. respectively).

However, the cytogenetic findings of the present case revealed the reliability and the susceptibility of CVS for determination of mosaicism and suggest the consideration of a follow-up study, even with the presence of only two aneuploid mitoses in CVS.

Although, the risk of spontaneous abortion after performing CVS varies in differnt centers (1-4%) , it is also important to consider the maternal age which causes an increases in the abortion rate, and also other environmental factors. The spontaneous abortion in women over 35 years of age is 4.0 - 4. 3% (4,15), however, among the present cases no spontaneous abortion occured due to CVS.

50% of the spontaneous abortion are caused by chromosome aberrations of the concceptus. About 1 in 400 newborns is reported to have structural chromosome aberrations including one-quarter with unbalanced translocations (16). However, cytogenetic investigation, as early as possible, on CVS, is a workable and reliable diagnostic tool for prenatal genetic diagnosis and genetic counselling.

The preventive approach of CVS has its importance for prenatal diagnosis in Iran. The ultimate result of routine prenatal diagnosis by CVS will be "a healthy life and healthy infants".

ACKNOWLEDGEMENT

The author is indebted to Dr. Nasseri, Dept. of Obstetrics and Gnycology, Pars Hospital, Tahran for the biopsy of Chorionic Villus Samples.

Table I: The Distribution of the Cases

20 Go.	10	38	46,XX			Girl- confirmed
21 M.Z	9	40	46,XX			Girl- "
22 Kn.	13	36	46,XY	Placenta previa		Boy- "
23 D.	$8^{1/2}$	40	46,XY			Boy "
			2 cells with 42,XY,+21 karyotype			Boy(Mosaic) "
						Girl "
24 khe	11	31	46,XX 1 cell with 47,XX,+8 karyotype			Girl "
25 Ro	12	42	46,XX			
26 S.	12	38	46,XX	Fibromatosis & uterus bleeding		Girl-confirmed
27 Gh.	12	28	46,XX		1 case of Down's in family	Girl "
28 Da.	9	35	46,XY incl. ctb. at lp; lcen.; 2p; 2q.	Previous pregnancy with Down's		Deceased
29 A.	9	39	Twin(polyploid)			1 fetus spontanously aborted
30 Jo.	10	36	Inadequate sample			Continuation of pregnancy
31 H	11	39	46,XX			" "
32 Ya.	11	40	46,XY			" "
33 Sar	10	44	46,XX			" "
34 Ra.	10	41	46,XX			" "
35 Ho.	10	35	46.XY			" "
36 Am.	10	39	46,XY			" "

(Cont. Tanle 1)

No.& Ref.	Gestational age (weey)	Maternal age(Year)	Cytogenetic Findings	Complications	History of Previous abortio(S)	Birth
1 Ki.	9	35	46,XX			Girl-confirmed
2 H	10	42	46,XX 1 cell with endoreduplication			Girl "
3 M.M.	10	42	46,XX	Posterior placenta & Bleeding		Girl "
4 Ka.	12	36	46,XY			Boy- "
5 T.	10	33	46,XX			Girl
6 B.	$13^{1/2}$	37	46,XY			Boy- "
7 Si.	12	37	Inadequate sample		A case of Down's in family	Boy- "
8 R.	13	43	Inadequate sample	(Late weeks' gestation)		
9 A.	10	38	Few cells,no mitosis			
10 So.	11	42	46, XY			Boy - confirmed
11 Sa.	14	40	46,XX(few mitosis)			Girl- "
12 F.	9	42	46,XX			Girl- "
13 M.	9.	31	46,XY		2 cases of Down's in family	
14 Gh.M.	10	41	46,XY			Boy- "
15 Mo.	12	38	No mitosis			
16 Fa.	12	40	Indequate sample			
17 J	11	34	46,XY			Boy -confirmed
18 R	$9^{1/2}$	29	46,XX		1 case with MR. in family	Girl "
19 GH.	$10^{1/2}$	37	46,XY			Boy- "

Table 2- Cytogenetic findings of 30 CVS (Spontaneous aborted materials in Iran).

No. of samples (%)	Weeks' Gestation	cytogenetic findings
12(40%)	7-13	46,XX
10(33%)	7-13	46,XY
1(3.3%)	7	47,XX,+21
1(3.3%)	8	47,XY,+18
6(16.6%)	8-12	No mitosis
Total : 30	7-13	24(80%) successful growth

Table 3- Cytogenetic findings of 36 CVS in Iran

No. of samples (%)	Weeks ' Gestation	cytogenetic findings
18(50%)	8-14	46,XX
11(30.5%)	8-13	46,XY(incl.a case with two trisomic cells: 47, XY,+21)
1 (2.7%)	9(Twin)	polyploid
2 (5.5%)	10 & 12	No mitosis
4 (11.1%)	10 & 12 & 13	Inadequate samples
Total: 36	8-14	29(80.5%) successful growth

REFERENCES

1. Benn, P.A.; Hsu, L.Y.; perlis, T., Schonhaut, A. (1984). prenatal Diagnosis of Chromosome Mosaicism. Prenatal Diag. 4: 1-9.

2. Cheung S.W., Crane J.P., Kyine M., Cui, M.Y. (1987) Direct Chromosome preparations from Chorionic Villi: A Method for Obtaining Extended Chromosomes and Recognizing Mosaicism Confined to the Placenta. Cytogenet. Cell Genet. 45: 118-120.

3. Drugan, A., Greb, A., Johnson, M.P., krivchenia, E.L.; Ulmann, W.R., Moghissi, K.S. and Evans M.I.(1990) Determinations of Parental Decissons to abort for Chromosome Abnormalities. Prenatal Diag. 10,8:483=490.

4. Gilmore, D.H. and Mc Nay, M.B. (1985) Spontaneous Fetal Loss Rate in Early pregnancy. Lancet I: 107.

5. Hsu, L.Y.F., kaffe, S. and Farlis, T.E. (1991) A Revisit of Trisomy 20 Mosaicism in Prenatal Diagnosis: An Overview of 103 Cases. prenatal Diag. ll,l: 7-15.

6. Jackson, L.G. (1985) CVS Latest News (Thomas Jefferson Univ. Philadelphia Feb. 2.

7. Kullander, S. and Sandahl, B. (1973) Fetal Chronmosome Analysis After Reancervical Prenatal Biopsy During Early Pragnancy. Acta Obstet Gynecol Scand 5: 355-359.

8. Martin, A.O., Simpson, J.L., Rosinsky, B.J., Elias S. (1986) Chorionic Villus Sampling Sampling in Continuing Pregnancies. II. Cytogenetic Reliability. Amer. J.Obstet. Gynenol. 154: 1353-1362.

9. Mehdipour, P., Nasseri, J. and Farhi, F. (1989)Prenatal diagnosis: Chorionic Villi Sampling (CVS). Shahid Beheshti, J. Fac. Mad. 12, 3 : 137-144.

10. Peckmann, D.C., Moreton, M.F., Corn, B.J., Robinson, A. (1978) Chromosomal Mosaicism in Amniotic Fluid Cell Cultures. Pediatrics Res. 12: 455.

11. Peckmann, D.C., Moreton, Moreton, M.F., Corn, B.J., Robinson, A. (1979)Chromosomal Mosaicism in Amniotoc Fluid Cell Cultures. Am. J. Hum. Genet. 31: 149-155.

12. Simoni,G., Brambati, B., Danesino, C., Terzoli, G.L., kossela, F. and Fraccaro, M.

(1994) Diagnostic Application of First Trimester Trophoblast Sampling in 100 pregnancies. Hum. Genet 66: 252-259.

13. Verjaal M., Leschot, N.J. , Wolf, H.; Treffers, P.E. (1987) Karyotypic Differences Between Cells from Placenta and Other Fetal Tissues. prenatal Diag. 7:343-348.
14. Verkmans, M.j.j. and perry, T.B. (1986) cytogenetic Analysis of Chorionic Villi. A Technical Assessment. Hum. Genet. 72: 307-310.
15. Wilson, R.D., kendrick, V., Wittmann, B.K. and pregnancy Outcome After Normal First Trimester Ultrasound Examination. obstet. Gynecol. 67:352-255.
16. World Health Organization working Group (1984) Fetal Diagnosis of Hereditary Disease. Bull. WHO 62:345-355.