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Prevalence of Chromosomal Abnormalities by Genetic Amniocentesis for Prenatal Diagnosis at Rajavithi Hospital : 1999-2002

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ABSTRACT

Objective To evaluate results of amniocentesis for prenatal diagnosis of chromosome abnormality at Rajavithi Hospital.

Study Design Retrospective descriptive study.

Setting Perinatal unit, Department of Obstetrics and Gynecology, Rajavithi Hospital.

Subjects One thousand four hundred and six patients who had genetic amniocentesis at perinatal unit, Department of Obstetrics and Gynecology, Rajavithi Hospital from January 1, 1999 to December 31, 2002.

Methods Records of mothers who had genetic amniocentesis during 1 January 1999 to 31 December 2002 were reviewed. Records of abnormal chromosome in newborn and results of genetic amniocentesis at Rajavithi Hospital were analyzed.

Results A total of 1,406 high risk pregnant women who underwent midtrimester genetic amniocentesis at Rajavithi Hospital were studied during January 1, 1999 to December 31, 2002. The most common indication was elderly gravidarum. Abnormal chromosome were detected prenataly 35 (2.5%). Women with chromosome abnormalities fetus underwent termination of pregnancy 22 (62.9%). There were 21 (1.6%) spontaneous abortion in normal karyotyping. Cell culture failure was 25 (1.8%). The cell culture failure was significantly increased in pregnancies with blood-stained amniotic fluid ($P < 0.001$).

Conclusion Genetic amniocentesis were highly successful performed with low complication. It is a safe and reliable method in prenatal diagnosis of chromosome abnormalities.

Key words: genetic amniocentesis, prenatal diagnosis , chromosomal abnormalities

Amniocentesis was first performed for genetic studies in the 1950s. Serr and colleagues[1], were the first to report the use of amniocentesis for antenatal sex determination. Prenatal diagnosis had its beginning in 1966[2], when Steele and Breg cultured amniotic cells and analyzed their karyotypes. Subsequently, amniocentesis has been performed for the diagnosis of a variety of disorders including chromosome abnormality. Midtrimester amniocentesis was established as the safe and accurate standard technique for prenatal diagnosis. In Rajavithi Hospital, we have been performing amniocentesis since 1995. This report analyzed the prevalence of abnormal fetal chromosome by cell culture of the amniotic fluid

in high risk pregnant women and pregnancy outcomes following amniocentesis.

Materials and Methods

In the 4 years period between January 1, 1999 and December 31 , 2002, 1,406 singleton women had a midtrimester amniocentesis at Rajavithi Hospital. After process of counseling and signing consent form. The Obstetrician staff or the 2nd or 3rd year resident will use ultrasound guidance to pass a 20 gauze spinal needle into the amniotic sac while avoiding trauma to the placenta, umbilical cord, and fetus. The initial aspirate of 1 ml of fluid is discarded to decrease the chance of maternal cell contamination and then approximately 20 ml of fluid is collected. After the procedure ultrasound examination was repeated to confirm fetal heart motion. Our laboratory used Ham F-10 media for culture amniocytes. Amniocytes were cultured in two different flasks. After harvesting and spreading the chromosome on the slides, we used Trypsin and Geimsa staining technique for banding. The chromosome were analyzed for 15-25 metaphases, then photographed and karyotyped for 2 metaphases. The women were followed until delivery. The patients who loss follow up will be called by telephone or mail to collect the missing data. The results of fetal karyotype, pregnancy outcome and complications which recorded in amniocentesis register form and medical record were identified. Every baby was examined grossly by pediatrician. For economic reason, we do not confirm chromosome study in every child birth. We used Pearson Chi - square for analysis qualitative data and percentage for quantitative data.

Results

A total of 1,406 amniocentesis was performed during the study period. The numbers of genetic amniocentesis has been increasing every year from 330 in 1999 to 370 in 2002 as shown in Table 1. The indications for amniocentesis are shown in Table 2, the most common indication for amniocentesis was advanced maternal age (94.1%). There were 1,029 amniocentesis, representing 73.2 % (1209/1406) of the total, performed during 16-18 wk, the patient distribution by gestational age is shown in Table 3. The distribution of women ages were shown in Table 4, the most common age group were 35-39 years (76.7%). Most of amniotic fluid specimens were successfully obtained at first attempt (98.2 %). Over-all fail cultured rate was 25 (1.8%). In 25 cases of culture failure, 23 cases had repeated amniocentesis. The results of abnormal chromosome are shown in Table 5 and figure 1. There were overall 2.5% (35/ 1404) of abnormal chromosomes, 0.6 % with structural, 1.6% with autosomal trisomies and 0.3% with sex chromosome aneuploidy. All case with structural chromosomal abnormalities decided to continue pregnancy, 91 % autosomal trisomies decided to terminate pregnancy and 50% sex chromosome abnormalities decided to continue pregnancy. Factors affecting failure of cell-culture are shown in Table 6, there is significantly increased risk of cell- culture failure in blood-stained amniotic fluids. (P-value< 0.001). Factors affecting abortion are shown in Table 7, the number of needle insertions, site of needle insertion, operator's experience and color of amniotic fluid were not significantly increase abortion rate (P-value>0.05). However, we had adequate data for analysis in only 1,302 of these women. The complication of amniocentesis are shown in table 8. We found spontaneous abortion rate was 1.6% and death fetus in utero rate was 0.1 %. In death fetus in utero case, the fetus was dead after performing amniocentesis 12 weeks. Although we cannot prove that it was the results of amniocentesis, we included this data in complication group. No other serious complications related to amniocentesis was found, for example: rupture of membrane, fetal injury.

Table 1. Numbers and results of amniocentesis by year.

Year	Genetic amniocentesis	Chromosome abnormalities	Percent
1999	330	9	2.7
2000	335	12	3.6
2001	369	8	2.2
2002	370	6	1.6
Total	1404	35	2.5

Table 2. Indication of amniocentesis.

Indication	Number	Percent
1. Elderly Gravidarum	1323	94.1
2. Maternal anxiety	28	2.0
3. Familial Chromosomal Disorder	18	1.3
4. Abnormal sonographic finding	14	1.0
5. Previous Down Syndrome	5	0.4
6. Previous child of mental retard	2	0.1
7. Habitual Abortion	1	0.1
8. Polyhydramnios	1	0.1
9. Others	14	1.0
Total	1406	100.0

Table 3. Gestation age at time of amniocentesis

GA[Week]	Number	Percent
< 16	6	0.4
16-18	1029	73.2
19-21	340	24.2
22-24	23	1.6
>24	8	0.6
Total	1406	100.0
Mean GA $\pm$ SD	17 $\pm$ 1.6	

Table 4. Result of amniocentesis by maternal age distribution.

Ages [Yrs]	Number	Percent
<30	26	1.8
30-34	55	3.9
35-39	1078	76.7
>39	247	17.6
Total	1406	100.0
Mean Age $\pm$ SD	36.8 $\pm$ 2.6	

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>39	247	17.6
Total	1406	100.0
Mean Age ± SD	36.8±2.6	

Table 5. Results of abnormal chromosome and fetal outcome.

Abnormal Karyotypng	Outcome			
	Induced abortion	Delivery	Continuous Pregnancy Mode of Delivery	BW
*** Structural abnormalities**				
46,XY,inv(9)(p12,q12)		Mature	C/S	3650
46,XX,inv(9)		Mature	Vg	3550
46,XX,16qh+		DFIU	Vg	2100 (true knot)
46,XY,16qh+		Mature	C/S	2960
46,XX,18p+qr46,XXder(18)		Mature	C/S	2500
46,XX,22p+		Mature	C/S	2650
464 46,XY,t (15:20)(q14:13)		Mature	C/S	3850
46,XX,t (1,17)(p21,p21)		Mature	C/S	3285
46,XY,t (5,16)(p15,q22)		Mature	C/S	3750
*** Numerical abnormalities**				
- Sex chromosome				
47,XXX		Mature	C/S	2500
47,XYY		Mature	Vg	3300
47,XYY	1			
69,XXX	1			
- Autosome				
47,XX,+13	1			
47,XY,+13	2			
47,XX,+18	2			
47,XY,+18	3			
47,XX,+21	3			
47,XX,+21		Mature	C/S	3760
47,XY,+21	8			
47,XY,+21		Mature	Vg	3200
46,XY/47,XY+21	1			

Table 6. Factors affecting failure of cell- culture.

Factors	Culture success	Culture failure	P - value *
1. Site			0.307
- placenta penetration	180 (97.3)	5 (2.7)	
- no	1201 (98.4)	20 (1.6)	
2. Obstetrician			0.283
- Resident	679 (97.8)	15 (2.2)	
- Staff	702 (98.6)	10 (1.4)	
3. Number of tap			0.968
-1 tap	1329 (98.2)	24 (1.8)	
- >1tap	52 (98.1)	1 (1.9)	
4. Color			<0.001
- Clear	1358 (98.5)	20 (1.5)	
- Blood-stained	23 (82.1)	5(17.9)	

* Pearson Chi -square

Table 7. Factors affecting abortion.

Factors	No	Abortion	P - value *
1. Site			0.071
- placenta penetration	173 (100)	-	
- no	1108 (98.1)	21 (1.9)	
2. Obstetrician			0.351
- Resident	612 (98.7)	8 (1.3)	
- Staff	669 (98.1)	13 (1.9)	
3. Number of tap			0.351
- 1 tap	1234 (98.5)	19 (1.5)	
- >1 tap	47 (95.9)	2 (4.1)	
4. Color			0.663
- Clear	1256 (98.4)	21 (1.6)	
- Blood-stained	25 (100.0)	-	

* Pearson Chi-square

Table 8. Complication of amniocentesis (N =1302).

Complications	Number	Percent
Spontaneous abortion	21	1.6
DFIU	1	0.1
AF leakage	0	0
Fetal injury	0	0
Total	22	1.7

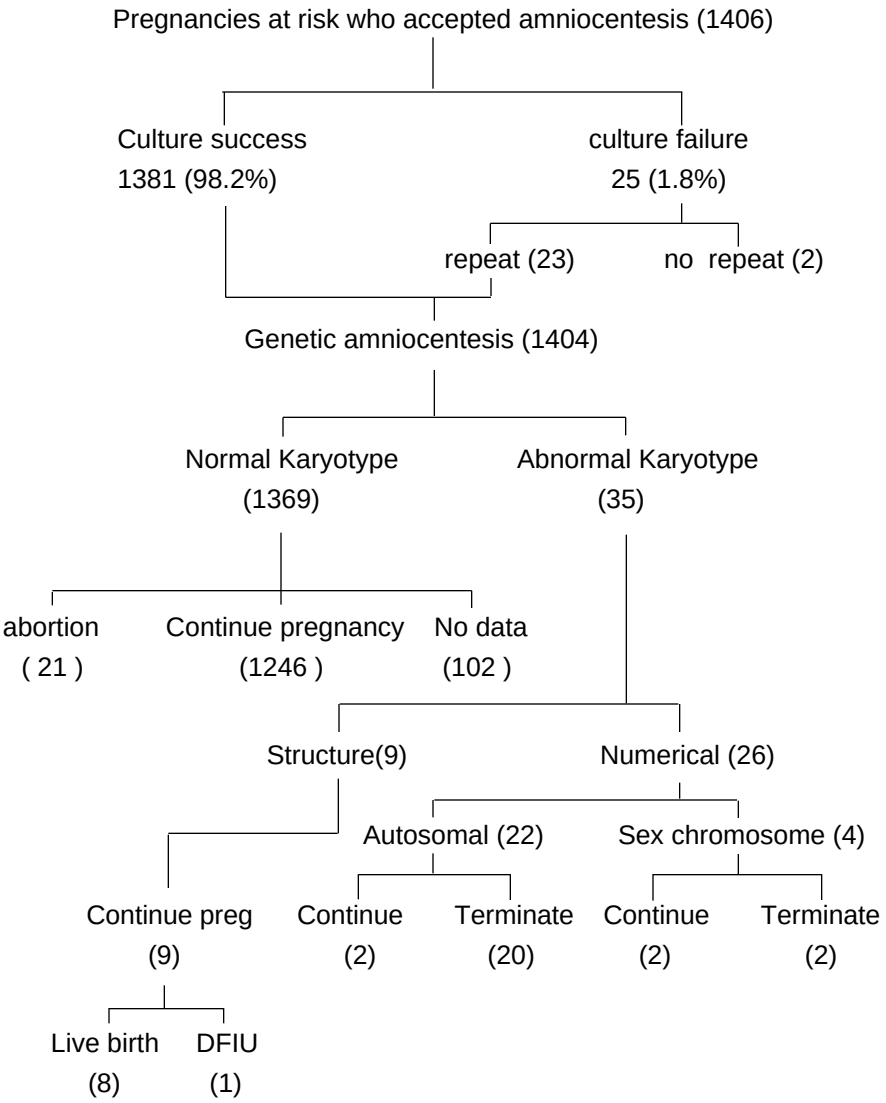

Fig . 1. Summary of the results of chromosome study

Discussion

Prevalence of chromosome abnormalities in this study had no difference from the others.[9] All of the cases of structural chromosomal abnormalities were determined to continue pregnancy because we informed the patients that most of them were variation and no evidence of mental retardation reported in scientific data.[3] No major abnormality was found among this group. Meanwhile, in the cases of numerical autosomal abnormalities, e.g. trisomy 13, most of couple decised to terminated because of the poor outcome including mental retardation or multiple organ anomalies reported in scientific literatures.[3] Prenatal counseling was essential and information should be given in detail especially in case of sex chromosome aneuploidy because these babies could survive to adulthood. Despite appropriate counseling, half of them were terminated by the decision of couples.

Several studies have confirmed that the incidence of bloody taps, amniotic fluid leakage and numbers of tapping are inversely related to the operator ùs experience. In the Netherlands study,[4]

Leschot et al found a fetal loss rate of 1.53% for the first 1,500 cases and 0.47% for the remaining group. Kappel et al reported the risk of spontaneous abortion was significantly increase both in pregnancies which the placenta was penetrated and in those with blood-stained amniotic fluid.[5] Complication related to prenatal diagnosis utilizing amniocentesis are abortion, death fetus in utero, leakage of amniotic fluid and direct fetal injury.

The amniocentesis group had spontaneous abortion rate 1.6 %, death fetus in utero of 0.1%, which was not significantly different from other studies.[7,8] The number of tapping, the site of tapping, operator's experience and color of amniotic fluid did not statistically increase abortion rate which were difference from the other studies.[5]

Our complication are higher than some studies because our hospital are training hospital and most of our patients are low socioeconomic and have to go back to work early after amniocentesis.

In this study, most of the amniotic fluid were successfully obtained at first attempt (98.2%) Overall fail cultured rate was 1.8% which was the same as other studies.[6] There is statistically increased in cell culture-failure in blood-stained amniotic fluid. High culture failure rate may be from fungal infection, blood contamination in amniotic fluid, advance gestational age, chemical culture media reagent and amniocentesis technique. Our laboratory uses Ham F-10 which is not expensive for culturing amniocytes. The more suitable reagent is Chang media because it can increase colonies of cell culture but is more expensive for government hospital. The other causes of culture failure have to be proved by fungal culture or need more data which are limited in this study.

Another limitation of this study, was that some cases could not be followed up to evaluate pregnancy outcomes because they gave birth at other hospitals, despite our attempts to collect all data.

In conclusion ,amniocentesis is still a safe method in prenatal diagnosis of chromosomal abnormalities. However, we should inform the couples about amniocentesis complications, amniotic culture failure and baby outcome especially baby with abnormal chromosome.

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