Midtrimester Amniocentesis for Antenatal Diagnosis of Genetic Disorder : Chulalongkorn Hospital Experience

Somchai Suwajanakorn MD,*
Yuen Tannirandorn MD,*
Orasri Romayanan MD,**
Sukhit Phaosavasdi MD.*

*Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University.
**Department of Anatomy, Faculty of Medicine, Chulalongkorn University.

Abstract : *Results of amniocentesis for prenatal diagnosis at Chulalongkorn Hospital have been studied during June 1991 to May 1992. A total of 250 pregnant women with 261 amniocentesis fluid samples were included in this study. Most of the indications are maternal age older than 35 years. The incidence of chromosomal abnormality detection in the mother older than 35 years of age is 13.57/1000 amniocenteses, while no chromosomal abnormality detected in mother less than 35 years of age. We further analyse the two methods used for amniocentesis, one with ultrasound guidance and the other with ultrasound located at the site of puncture. The two methods did not differ in blood contamination in amniotic fluid nor the number of punctures. Also there was no abortion related to both procedures. The cost analysis for performing amniocentesis in the mother age beyond 35 is very reasonable according to our study. In conclusion, amniocentesis is a safe method for prenatal chromosome diagnosis and recommended for mothers who are older than 35 years old. (Thai J Obstet Gynaecol 1994;6:43-49.)*

Key words : amniocentesis, antenal diagnosis genetic disorder

Following the successful culturing of fetal cells from amniotic fluid by Steele and Breg[1] in 1966, midtrimester amniocentesis was established as a safe and accurate standard technique for prenatal diagnosis[2,3]. It has become a routine prophylactic examination, in developed countries, offered to women with an increased risk of having a child with a chromosome abnormality, neural tube defect or metabolic disease[4,5]. In Thailand, those who are at risk for having a chromosome abnormality fetus will be advised for midtrimester amniocentesis but not all of them have

been done due to the inavailability of genetic laboratory and inexperience of obstetricians. In Chulalongkorn Hospital, we have been performing amniocentesis since 1978 but few cases were done weekly at that time. Nowadays, with the development of high-resolution ultrasonography that makes the amniocentesis much safer, a program of midtrimester amniocentesis for antenatal diagnosis of genetic disorder has been set up with the aim of promoting obstetricians to refer the high risk group patients for amniocentesis.

The purpose of the present study is to assess the frequency of chromosome abnormalities in high risk pregnant women, the safety of the method and to evaluate the cost benefit for this program.

Materials and Methods

During June 1991 to May 1992, 250 women had chromosome analysis performed on 261 consecutive samples of amniotic fluid (6 pairs of twins). The indication of amniocentesis is shown in Table 1. Transabdominal amniocentesis was performed between 14-22 weeks gestation. When the date was uncertain, ultrasonography was performed earlier to confirm gestational age. During that period, amniocentesis was carried out by two methods : one by under ultrasonic guidance (UG) and the other by locating the largest pocket of amniotic fluid by ultrasound and then punctured by free hand technique (UL) : the first two authors. Since 1989, we have used linear real time ultrasound scanning (Hitachi, model EUB-40). In twin pregnancies, sampling from both fetal sacs were applied. To avoid maternal cell contamination, the first 2 mls. of amniotic fluid were aspirated and discarded in a separate syringe. Fetal chromosome analysis was carried out using trypsin -G - banding technique[6]. Using Chi's square to compare the statistical significant difference between outcome of the two methods used for amniocentesis and Chi's square with Yate correction where it is necessary.

Results

Of the 250 women performed amniocentesis at our hospital during the study period, 5 (1.95%) had to have repeat amniocentesis due to culture failure which might partly respond by bacterial contamination, transportation technique or culture

Table 1 *Indication*

Indication	
Elderly	212
IVF	11
Previous Chromosome abnormality	7
Family History of Chromosome abnormality	6
Habitual abortion	4
others	10
Total	250

Table 2 *Age Distribution by gestation*

Gestation / Age	1	2	3	4	5	6	7	Total
20-24	1	3	-	2	-	-	-	6
25-29	1	-	4	-	-	-	-	5
30-34	8	5	3	2	-	-	-	18
35-39	52	63	32	21	6	3	-	177
>40	11	14	10	7	-	1	1	44
Total	73	85	49	32	6	4	1	250

Table 3 *Results of Amniocentesis*

Chromosome	N
46 xx	127
46 xy	126
47 xx + 21	1
47 xx + 21	1
47 xxy	1
Total	256*

* = 6 pairs of twins

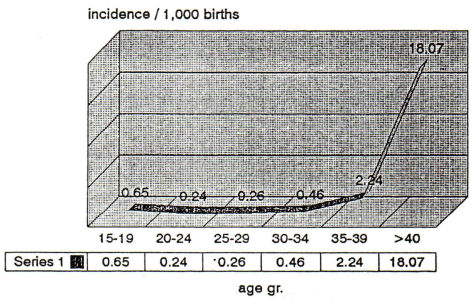

Figure 1 Incidence of chromosome abnormality and age of the mother who delivered at Chulalongkorn Hospital. (June 1991 - May 1992)

technique failure. Most of the indication (84.8%) (Shown in Table 1) was elderly gravida. The distribution of the women's age shown in Table 2, 221 (88.4%) were more than 35 years of age. Cytogenetic results and incidence of chromosomal abnormality are shown in Table 3 and 4, Figure 1. The number of amniocentesis using 2 different techiques are shown in Table 5. Some information was not available so only 243 punctures were included in this study. The results of both procedures are shown in Table 6 and 7. No abortion related to the procedure was found in our study.

Usually the cost of amniocentesis at Chulalongkorn Hospital is 2,500 - 3,000 bahts for a case. (These include the cost of ultrasound, amniocentesis procedure and cell culture, We have 221 mothers age beyond 35 who had amniocentesis during the study period. Three of them have

Table 4 ***Resultsof Amniocentesis***

	No. of Mother *	No. of Amniocentesis	No. Revealing Chromosome Abnormality.	No. of Chromosome Abnormality	Incidence (1: 1000)
≤14	15	-	-	-	
15-19	1536	-	-	1[A]	0.65
20-24	4090	6	-	1[A]	0.24
25-29	3845	5	-	1[A]	0.26
30-34	2176	18	-	1[C]	0.46
35-39	894	177	1[A]	1[A]	2.24
≥40	166	44	2[A, B]	1[A]	18.07
Total	12,722	250	3	6	

* = Number of mothers who delivered at Chulalongkorn Hospital
A = Trisomy 21
B = 47 xxy
C = 45 xo

Table 5 ***Method of Amniocentesis***

Ultrasonogram Guided	206
Ultrasonogram Located	37
Total	243

Table 6 ***Number of puncture related to method of ammiocentesis***

No Puncture / Method Of Ammiocentesis	1	2	3	Total
Ultrasonogram Guided	200[A] (97.1)	6 (2.9)	0 (0)	206 (100)
Ultrasonogram Located	33[A] (89.2)	3 (8.1)	1 (2.7)	37 (100)
TOTAL	233 (95.9)	9 (3.7)	1 (0.4)	243 (100)

A (P = 1.5091)

Table 7 *Amniotic Fluid (AF) colour related to amniocentesis through placenta*

AF COLOUR \ THROUGH PLACENTA	YES	NO	Total
CLEAR	91[A]	141[A]	232
BLOOD	7	4	11
TOTAL	98	145	243

A (P=0.040884)

abnormal chromosomes. The detection of one case of chromosome abnormality fetus will be simply calculated as followed :

Number of amniocentesis cases age beyond 35 years =221
Amniocentesis cost =3,000 Bhts
The total cost for amniocentesis =663,000 Bhts
Chromosome abnormality detected =3
Cost for one abnormality detected =221,000 Bhts

Discussion

The major cytogenetic indication for amniocentesis are advanced maternal age and chromosome abnormality in a previous child, conceptus, or family history of a chromosome abnormality child which contributes about 90% of all cases. Our study of cytogenetic results of 256 amniotic fluid specimens showed three abnormal karyotypes which were two trisomy 21 and one 47 XXY. Our rates for detection of chromosome abnormalities through amniocentesis according to single-year intervals of maternal age beyond 35 years were 13.57/1000. There was no chromosome abnormality detected from amniotic fluid specimen of women under age 35 years. However, six chromosomes abnormal offsprings were born after mothers of low risk or late antenatal care in the same period of our study at Chulalongkorn Hospital. Five of whom were trisomy 21 and the other was 45 XO. Of these, 4 chromosome abnormal infants were born after mother of less than 35 years of age. When considering the total number of chromosome abnormality fetuses and infants born after the mother who attended our antenatal clinic, we found that at age below 35 years, the prevalence of chromosome abnormality was 0.34/1000 pregnant women while at age 35 years up, the prevalence was 3.90/1000 pregnant women which was 10 times more than age below 35 years.

Since we have two techniques of amniocentesis : UG and UL, we further analyze the safety of each

method by comparing mainly the color of AF. The result shows that UG technique has a tendency to obtain less bloody amniotic fluid than UL technique, however, there is no statistical significant difference between these two techniques. Also, the number of percutaneous punctures between these two techniques have no statistical significant difference. These effects may be due to the small number of studied population and in part by the current experience of the authors who performed amniocentesis which need no accurate guiding. Nevertheless, UG technique seems to be the best method in performing amniocentesis if the facility is available. In our series there was no abortion or fetal death related to the procedure.

Regarding the value of amniocentesis, especially mothers whose age are beyond 35, the cost for one detected chromosome abnormality fetus is 221,000 bahts which is reasonable in performing a chromosomal abnormality detected program since the total cost for caring of the chromosome abnormality is much higher than the cost in the program.

Conclusion

Among a population of 250 pregnant women, a total of 256 amniotic fluid specimens were obtained for fetal chromosome analysis. Most of the indication was elderly gravida. Of the amniotic fluid specimens analyzed, 2 were Down's syndrome and 1 was 47 XXY. The cost of analysis revealed the benefit for performing amniocentesis in mother older than 35 years of age. The technique of ultrasound guided and ultrasound located amniocentesis seemed not to be different in the result of yielding the amniotic fluid. However, we accepted that the ultrasound guided technique is safe and practical for performing amniocentesis.

Acknowledgement

This investigation was supported by the Rachadapiseksompoj China Medical Board Research Fund, Faculty of Medicine, Chulalongkorn Hospital.

References

1. Steele MW, Breg WR Jr. Chromosome analysis of human amniotic fluid cells. Lancet 1966;1:383-5.
2. National Institute of Child Health and Human Development Amniocentesis Registry, 1978. The safety and accuracy of mid-trimester amniocentesis. DHEW publication no. (NIH) 78-190. Washington DC : United State Department of Health, Education and Welfare, 1978.
3. Diagnosis of genetic disease by amniocentesis during second trimester of pregnancy. Ottawa, Canada : Medical Researh Council, 1977.
4. Tabor A, Philip J. Incidence of fetal chromosome abnormalities in 2264 low-risk women. Prenat Diagn 1987;7: 355-62.
5. Cruikshank DP, Varner MW, Cruikshank JE, Grant SS, Donnelly E. Midtrimester amniocentesis : an analysis of 923 cases with neonatal follow-up. Am J Obstet Gynaecol 1983;146:204-11.

6. Harrison CJ. Diagnosis of malignancy from chromosome preparation. In : Rooney DE, Czepulkowski BH, eds. Human Cytogenetics : a practical approach. Oxford : Information Printing, 1986 : 152.