Thai Journal of Obstetrics and Gynaecology
December 1999, Vol. 11, pp. 209-216

## OBSTETRICS

# Genetic Amniocentesis for Prenatal Diagnosis at Pramongkutklao Hospital: A six-year report

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## ABSTRACT

**Objective** To evaluate results of amniocentesis for prenatal diagnosis of chromosome abnormality and analyze Down syndrome controlling program using maternal age cut-off at Pramongkutklao Hospital.

**Method** Records of mothers who had genetic amniocentesis during 1 October 1990-30 September 1996 were reviewed. Records of abnormal chromosome in newborns and numbers of mothers who delivered at Pramongkutklao Hospital at the same period were also studied.

**Results** During six years, 28,191 women, including 2,174 (7.71%) elderly gravida (>=35 years old), delivered at Pramongkutklao Hospital. 917 (3.25%) amniocentesis were done from 906 mothers (11 twin pregnancy) from the following indication: 1) elderly gravida 2) previous chromosome disorder 3) fetal malformation 4) familial history of chromosome disorder 14 (1.53%) major abnormal chromosomes were detected prenatally. 16 (1.74%) samples failed to culture. The indication that most commonly found major abnormal chromosome was fetal malformation (4/18), but no major abnormal chromosome was found from the Indication of previous chromosome abnormality and familial history of chromosome disorder. Most common major abnormal chromosome was Trisomy 21 (6/141). Our program to control incidence off Down syndrome using maternal age cut off at >=35 years old alone could access 782 (11 twin pregnancy) in 2,174 mothers (35.9%) and detect 5 Down syndrome fetus (5/793=1/159) and 3 other major abnormal chromosome fetus (8/793=1/99). Incidence of Down syndrome fetus and major abnormal chromosome born during this period were 0.89 and 1.56 /1,000 live births respectively. The program could detect 5/30 (16.6%) of Down syndrome fetus and 8/52(15.4%) of all major abnormal chromosome fetus.

**Conclusion** Elderly mothers were increasing. Although there was an increasing access to elderly mothers of the controlling program, the incidence of Down syndrome in our hospital did increase. Genetic counsellors, new methods of screening and confirmatory test as well as improvement of cytogenetic lab will be required in the near future.

**Key words:** prenatal diagnosis, amniocentesis, Down syndrome

Amniocentesis was first performed for genetic studies in the 1950. At the beginning, It used only for antenatal sex determination.[1] In 1966, prenatal diagnosis had begun, when Steele and Breg[2] cultured amniotic cells and analyzed their karyotypes. Subsequently, amniocentesis has been performed for the diagnosis of variety disorders including chromosomal abnormality. In developed countries, it has been offered to women with an increased risk of having a child with chromosome abnormality, neural tube defect or metabolic disease.

Pramongkutklao Hospital has established the program of genetic counseling and prenatal diagnosis with midtrimester amniocentesis since 1985. The program was aimed to control the incidence of chromosome abnormality especially Down syndrome in live birth. Results of the first six years were studied by Ketupanya.[3] After that, the numbers of genetic amniocentesis has been increasing year by year, along with the numbers of elderly gravida. The purpose of this study is to evaluate the results of genetic amniocentesis in the following six years and analyzes Down syndrome controlling program using maternal age alone at Pramongkutklao hospital.

## Method

Records of women who had genetic amniocentesis during 1 October 1990-30 September 1996 were reviewed. Records of abnormal chromosome neonates and women who delivered at Pramongkutklao hospital during the same period were studied.

Program of genetic counseling and prenatal diagnosis at Pramongkutklao Hospital

Elderly gravida (>=35 years old) who came to antenatal clinic during 16-20 gestational weeks were appointed to genetic clinic. After process of counseling and signing consent form, physicians performed ultrasonographic study to screen fetal anomaly and confirm dating. Amniocentesis was done and 30 cc of amniotic fluid was aspirated. After the procedure, ultrasound examination was repeated to confirm fetal heart motion. Amniocytes were cultured in three 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, photographed and karyotyped for 2 mataphases.

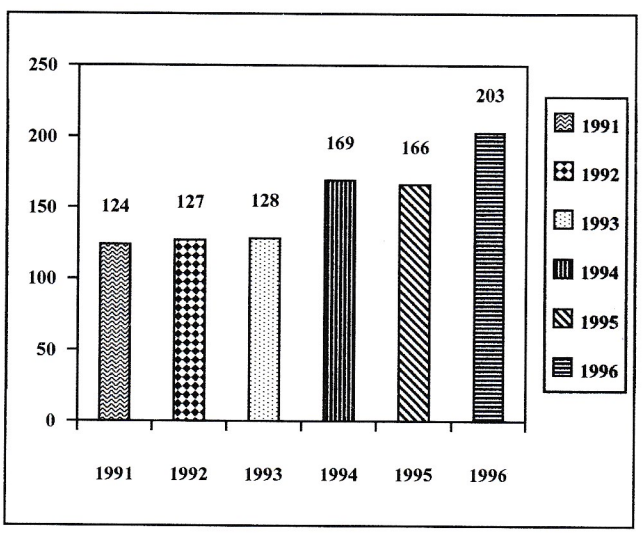


**Fig. 1.** Total numbers of amniocentesis by years.

**Table 1.** Numbers and results of amniocentesis by years

| YEARS | NUMBERS | RESULTS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NORMAL | FAIL | % | UNKNOWN | % | MAJ. ABN. | % | OTHER ABN | % |
| 1991 | 124 | 107 | 3 | 2.42 | 10 | 8.06 | 0 | 0.00 | 4 | 3.23 |
| 1992 | 127 | 123 | 0 | 0.00 | 3 | 2.36 | 1 | 0.79 | 0 | 0.00 |
| 1993 | 128 | 123 | 0 | 0.00 | 3 | 2.34 | 2 | 1.56 | 0 | 0.00 |
| 1994 | 169 | 160 | 0 | 0.00 | 3 | 1.78 | 4 | 2.37 | 2 | 1.18 |
| 1995 | 166 | 147 | 9 | 5.42 | 3 | 1.81 | 4 | 2.41 | 3 | 1.81 |
| 1996 | 203 | 194 | 4 | 1.97 | 1 | 0.49 | 3 | 1.48 | 1 | 0.49 |
| **TOTAL** | **917** | **854** | **16** | **1.74** | **23** | **2.51** | **14** | **1.53** | **10** | **1.09** |

**Table 2.** Results of amniocentesis by age distribution

| AGES | TOTAL No. OF DELIVERY | RESULTS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of Amnio | Maj. Abn. | | Other Abn. | | NORMAL | | FAIL | | UNKNOWN | |
| <20 | 3,175 | 5 | 0 | 0.00 | 1 | 20.00 | 4 | 80.00 | 0 | 0.00 | 0 | 0.00 |
| 20-24 | 8,542 | 13 | 0 | 0.00 | 0 | 0.00 | 10 | 76.92 | 1 | 7.69 | 2 | 15.38 |
| 25-29 | 9,056 | 52 | 2 | 3.85 | 1 | 1.92 | 46 | 88.46 | 0 | 0.00 | 3 | 5.77 |
| 30-34 | 5,244 | 51 | 4 | 7.84 | 2 | 3.92 | 43 | 84.31 | 1 | 1.96 | 1 | 1.96 |
| 35-39 | | 640 | 5 | 0.78 | 6 | 0.94 | 604 | 94.38 | 13 | 2.03 | 12 | 1.88 |
| 40-44 | 2,174 | 152 | 3 | 1.97 | 0 | 0.00 | 144 | 94.74 | 1 | 0.66 | 4 | 2.63 |
| >44 | | 2 | 0 | 0.00 | 0 | 0.00 | 2 | 100.00 | 0 | 0.00 | 0 | 0.00 |
| UNKNOWN | | 2 | 0 | 0.00 | 0 | 0.00 | 1 | 50.00 | 0 | 0.00 | 1 | 50.00 |
| **TOTAL** | **28,191** | **917** | **14** | **1.53** | **10** | **1.09** | **854** | **93.13** | **16** | **1.74** | **23** | **2.51** |

**Table 3.** Results of amniocentesis by indications

| INDICATIONS | NUMBERS OF AMNIO. | RESULTS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NORMAL | | MAJ. ABN. | | OTHER ABN. | | FAIL | | UNKNOWN | |
| 1. AGE 35-37 | 427 | 406 | 95.08 | 4 | 0.94 | 3 | 0.70 | 7 | 1.64 | 7 | 1.64 |
| 2. AGE >=38 | 366 | 343 | 93.72 | 4 | 1.09 | 3 | 0.82 | 7 | 1.91 | 9 | 2.46 |
| 3. PREV. CHRO. ABN. | 28 | 26 | 92.86 | 0 | 0.00 | 2 | 7.14 | 0 | 0.00 | 0 | 0.00 |
| 4. MALFORMATION | 18 | 13 | 72.22 | 4 | 22.22 | 0 | 0.00 | 1 | 5.56 | 0 | 0.00 |
| 5. FAMILIAL HX | 8 | 7 | 87.50 | 0 | 0.00 | 1 | 12.50 | 0 | 0.00 | 0 | 0.00 |
| 6. OTHER | 55 | 52 | 94.55 | 2 | 3.64 | 1 | 1.82 | 0 | 0.00 | 0 | 0.00 |
| 7. UNKNOWN | 15 | 7 | 46.67 | 0 | 0.00 | 0 | 0.00 | 1 | 6.67 | 7 | 46.67 |
| **TOTAL** | **917** | **854** | **93.13** | **14** | **1.53** | **10** | **1.09** | **16** | **1.74** | **23** | **2.51** |

**Table 4.** Major chromosome abnormalities

| TYPES OF ABNORMALITY | NUMBERS |
|---|---|
| 47, XX,+21 | 5 |
| 47, XY,+21 | 1 |
| 47, XY,+18 | 2 |
| 47, XX,+13 | 1 |
| 46, XX,t(13q 14q) | 1 |
| 45, XO | 1 |
| 45, XO/46, XX | 1 |
| 46, X, i(Xq)/45, XO | 1 |
| 47, XXY | 1 |
| **TOTAL** | **14** |

**Table 5.** Other chromosome abnormalities

| BALANCED TRANSLOCATION and INVERSION | |
|---|---|
| 1. TYPES OF ABNORMALITY | NUMBERS |
| 46, XX t(2, 18) BL translocation | 1 |
| 45, XY t(13q14q) BL translocation | 1 |
| **Total** | **2** |
| 2. MARKER CHROMOSOME | |
| 46, XX, INV (2)(p13, q11) | 1 |
| 47, XX, ISO 15p | 1 |
| **Total** | **2** |
| 3. NORMAL VARIATION | |
| 46, XX, 21 P+ | 1 |
| 46, XX, 15 P+ | 3 |
| 46, XX, 16 qh+ | 1 |
| 46, XX, 16 qh+ | 1 |
| | **6** |
| **TOTAL** | **10** |

**Table 6.** Abnormal chromosomes distributed by ages and indications

| NO. | TYPES OF ABNORMAL | MATERNAL AGES | INDICATIONS |
|---|---|---|---|
| | MAJOR ABNORMAL CHROMOSOMES | | |
| 1 | 47, XX, +21 | 36 | AGE 35-37 |
| 2 | 47, XX, +21 | 34 | ANXIETY |
| 3 | 47, XX, +21 | 37 | AGE 35-37 |
| 4 | 47, XX, +21 | 37 | AGE 35-37 |
| 5 | 47, XX, +21 | 42 | AGE >=38 |
| 6 | 47, XY, +21 | 42 | AGE >=38 |
| 7 | 47, XY, +18 | 39 | AGE >=38 |
| 8 | 47, XY, +18 | 26 | DIL.CISTERNA MAGNA, CLENCH HAND |
| 9 | 47, XX, +13 | 32 | HOLOPROSENCAPHALY |
| 10 | 46, XX,-14,+t (13q14q) | 26 | HOLOPROSENCAPHALY |
| 11 | 45, XO | 34 | CYSTIC HYGROMA |
| 12 | 45, XO / 46, XX | 37 | AGE 35-37 |
| 13 | 46, X, i (Xq) / 45, XO | 34 | ANXIETY |
| 14 | 47, XXY | 43 | AGE >=38 |
| | OTHER ABNORMAL CHROMOSOMES | | |
| 15 | 46, XX t (2,18) BL translocation | 26 | TRANSLOCATION MOTHER |
| 16 | 45, XY, t (13q, 14q) BL translocation | 30 | TRANSLOCATION FATHER |
| 17 | 46, XX, inv (2)(p13 q11) | 31 | AGE 35-37 |
| 18 | 47, XX, iso 15p | 38 | AGE >=38 |
| 19 | 46, XX, 21 p+ | 30 | PREVIOUS ANENCEPHALY AND ANXIETY |
| 20 | 46, XY, 15p+ | 39 | AGE >=38 |
| 21 | 46, XY, 15p+ | 39 | AGE >=38 |
| 22 | 46, XY, 15p+ | 37 | AGE 35-37 |
| 23 | 46, XX, 16qh+ | 36 | AGE 35-37 |
| 24 | 46, XX, 16qh+ | 19 | PREVIOUS CHROMOSOME ANOMALY |

**Table 7.** Analysis of Down's syndrome controlling program using maternal ages alone

| YEARS | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | TOTAL |
|---|---|---|---|---|---|---|---|
| NO. OF MOTHERS DEL. AT PMK HOSP. | 4374 | 4692 | 4742 | 4723 | 5020 | 4640 | 28191 |
| MATERNAL AGE >=35 | 289 | 359 | 369 | 364 | 370 | 423 | 2174 |
| RATIO TO ALL DEL. | 6.61% | 7.65% | 7.78% | 7.71% | 7.37% | 9.12%18 | 7.71% |
| NO. AMNIO. BY AGE INDICATION ALONE | 100 | 110 | 109 | 144 | 147 | 3 | 793 |
| RATIO TO ALL ELDERLY GRAVIDA | 34.60% | 30.64% | 29.54% | 39.56% | 39.73% | 43.26% | 36.48% |
| NO. DOWN DETECTED FROM AMNIO.* | 0 | 0 | 1(1/109) | 0 | 3(1/49) | 1(1/183) | 5(1/159) |
| NO. OF ALL MAJ. ABN. CHRO. FROM AMNIO.* | 0 | 0 | 1(1/109) | 2(1/72) | 3(1/49) | 2(1/91) | 8(1/99) |
| LIVE BIRTHS | 4387 | 4696 | 4689 | 4723 | 5034 | 4648 | 28177 |
| NO. OF DOWN AT BIRTH | 5 | 0 | 6 | 6 | 1 | 7 | 25 |
| DOWN INCIDENCE (/1000 LB) | 1.14 | 0.00 | 1.28 | 1.27 | 0.20 | 1.51 | 0.89 |
| NO. OF MAJ. ABN. CHRO. AT BIRTH | 8 | 4 | 9 | 9 | 5 | 9 | 44 |
| INCIDENCE OF MAJ. ABN. CHRO. (/1000 LB) | 1.82 | 0.85 | 1.92 | 1.91 | 0.99 | 1.94 | 1.56 |

* = BY AGE INDICATION ALONE

**Total 8.** Abnormal chromosomes found at birth with no prenatal diagnosis

| | MATERNAL AGES | | | | |
|---|---|---|---|---|---|
| | <35 | 35-37 | >=38 | UNKNOWN | TOTAL |
| DOWN SYNDROME | 8 | 5 | 3 | 9 | 25 |
| OTHER ABNORMAL CHROMOSOMES | 11 | 3 | 5 | 0 | 19 |
| TOTAL | 19 | 8 | 8 | 9 | 44 |

## Result

During the study period, The numbers of amniocentesis were increasing from 124 in 1991 to 203 in 1996 (Fig. 1). Total of 917 (3.25%) samples of amniotic fluid were drawn from 906 mothers 11 twins pregnancy). There were 24 abnormal chromosome, 14 (1.53%) were major abnormal chromosome, 10 (1.09%) were minor abnormal chromosome. 16(1.74%) samples were failed to culture. The results of 23 (2.51%) samples were missing (Table 1). During the same period, there were 28, 191 women who delivered at Pramongkutklao hospital. 2,174 (7.18%) were elderly gravida (>= 35 years old). Age of patients who had amniocentesis ranged from 17-45 (mean 36.3± 4.34). Out of 917 amniocentesis, 794 samples were done from 782 (35.9% of 2,174) elderly mothers (11 twins pregnancy) from the indication of age alone and another 1 elderly mother from the indication of malformed fetus. We could detect 8 major abnormal chromosome fetus prenatally from the elderly group and other 6 major abnormal chromosome fetus from the younger age group (Table 2). Indication for chromosome study were listed in Table 3. The most common indication that resulted in major abnormal chromosome was fetal malformation (4 in 18), but no major abnormal chromosome was found from the cases of previous chromosome abnormality and familial history of chromosome disorder. The numbers of, amniocentesis performed from indication of maternal aged 35-37 and aged >=38 were 427 and 366 respectively. Most common major abnormal chromosome was 21(6 in 14), the other were Trisomy 18, Trisomy 13, monosomy X, mosaic monosomy X and 47 XXY. (Table 4) The other minor abnormality were 3 balanced translocation and inversion, 1 marker chromosomes and 6 normal variations (Table 5). 5 Trisomy 21 and 3 other major abnormal chromosome were detected prenatally by aged indication alone (Table6). From this study, program to detect Down syndrome fetus using maternal age cut off at >= 35 years old, could access only 782 in 2,174 mothers (35.97%) and could detect 5 Down syndrome fetus (5 in 793 = 1/159) or 8 major abnormal chromosome fetus (8 in 793 = 1/99) from 793 samples. Incidence of Down syndrome and major abnormal chromosome fetus at birth during that period were 0.89 and 1.56/1000 live birth respectively. Therefore the program could detect only 5 in 30 (16.6%) of Down syndrome fetus and 8 in 52 (15.4%) of all major abnormal chromosome fetus prenatally (Table 7). When we analyzed 44 major abnormal chromosome fetuses born during the same period, there were 25 Down syndrome fetus and 19 major abnormal chromosome fetus. In the Down syndrome group, 8 were from mothers younger than 35 years old, 5 from mothers aged 35-37, 3 from mothers aged >= 38 and 9 from mothers whose age couldn't be identified. In the other major abnormal chromosome group, there were 11, 3, 5, 0 from the corresponding maternal age respectively. (Table 8)

## Discussion

Program of genetic counseling and amniocentesis in Pramongkutklao hospital has been established since 1985. The result of the program during 1985 to 1990 was presented by Ketupanya.[3] Since then, the numbers of genetic amniocentesis has been increasing every year from 124 in 1991 to 203 in 1996 (Table 1).

14 (1.53%) major abnormal chromosome from 917 samples were detacted prenatally. 10 (1.09%) other abnormal chromosome in this study refer to marker chromosome, balanced translocation, inversion and normal variation which have no significant clinical features. Overall fail cultured rate, was 1.74% which was the same as other studies (1-2%).[4] In 1995, 5% failed culture rate was from the problem of fungal infection in ventilatory system around cytogenetic laboratory room.

During six years, 28191 mothers, including 2174 (7.71%) elderly mothers, delivered at Pramongkutklao Hospital. We could perform 793 amniocentesis from 782 mothers (35.9%) when we used aged indication alone which was higher than access rate of Suwajanakorn's study in 1991-1992 (20.8%).[5] The reasons why we could not perform amniocentesis In all elderly mothers were late antenatal care, physician's negligence or patient's avoidance, which should be another issues to study further to increase the amniocentesis rate in our hospital.

Indication most commonly found abnormal chromosome was fetal malformation (4/18), but no abnormal chromosome was found from the indication of previous chromosome abnormality and familial history of chromosome disorder. This may be the result of small sample size (26 cases). Other indication in this study refered to previous neural tube defect, maternal anxiety and IVF pregnancies.

Most common major abnormal chromosome detected prenatally was Trisomy 21 (6/14), which was the same as other studies. The others were Trisomy 18, Trisomy 13, monosomy X, mosaic monosomy X and 47 XXY.

From the result catergorized by indication, when we used the maternal aged out off at >= 35 years old, we could detect 5 Down syndrome fetus ( 5 in 793 ~ 1/159 ) and 3 other major abnormal chromosome fetus (8 in 793 ~ 1/99) when performed 793 amniocentesis (table 3,6) Compared to the rate of 1/140 from other studies,[6] our program had a slightly lower rate of detecting Dawn syndrome fetus. If we used the cut off at >=38 years old, we could detect 2 Down syndrome fetus (2 in 366 = 1/1183) and 2 other major abnormal chromosomes for total detection rate of 1/90 (4 in 366) (table 3,6). The changing of the cut off age from 35 to 38 years old was debated during the last few years in our hospital. The cost effectiveness of both cut-offs had been estimated from the provider's view point in Ketupanya's study. They were 67,129 VS 36,617.7 Baht / case detection respectively.[3] However, from the parent's view point, we will miss 3 fetus of Down syndrome and another 1 abnormal chromosome fetus. This problem will burden their family and society eventually. This subject of debate came from the increasing numbers of elderly mothers and work load on cytogenetic lab.

With increasing numbers of elderly mothers who came to deliver at Pramongkutklao Hospital from 4,374 in 1991 to 5,020 in 1995 and the increasing proportion of elderly mothers from 6.61% in 1991 to 9. 12% in 1996, even we could increase access to elderly mothers from 34% in 1991 to 43% in 1996, the incidence of Down syndrome at birth did increase to 1.51/1000 live birth in 1996. (Table 6) When analyzed abnormal chromosome fetus born at the same period. if we had performed amniocentesis to all elderly mothers we could have detected other 8 Down syndrome and 8 other major abnormal chromosome fetus prenatally, but we still missed more than 8 Down syndrome and 11 other major abnormal chromosome fetus (Table 8). Therefore, new methods of screening and confirmatory test, increasing rate of access to elderly mothers, genetic counsellors as well as improvement of cytogenetic lab will be required in the near future in our hospital.

New markers to screen Down syndrome fetus especially the triple markers have been established in 1992.[7,8] Some suggest to use them to screen only mothers younger than 35 years old or to be another option for older women, who traditionally have all been considered candidates for amniocentesis. We will not incorporate them into our program until we can make sure that we can provide adequate pretest genetic counseling and our lab can manage with the increasing numbers of serum markers to be tested as well as

cytogenetic samples. Importantly, the cost effectiveness of the program in our population should be considered carefully.

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