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Second Trimester Genetic Amniocentesis: Khon Kaen University 14-year experience

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ABSTRACT

Objective: To evaluate the results of second trimester amniocentesis for prenatal diagnosis of chromosomal abnormalities at Khon Kaen University.

Material and Method: A review of data from 2,825 genetic amniocenteses between January 1, 1993 and December 31, 2006 at Srinagarind Hospital,Faculty of Medicine,Khon Kaen University. Data reviewed included medical records, amniocentesis record forms and questionnaires sent by mail that inquired about complications and pregnancy outcomes. The mail out questionnaires response rate was 75.5%.

Main outcome measure: Fetal loss rate after amniocentesis within 2 weeks.

Results: The most common indication for genetic amniocentesis was advanced maternal age (90.7%). Chromosomal abnormalities were found 3.1%. The most common chromosomal abnormality was trisomy 21 (0.91%). The procedure failed in only one case (0.03%). Additional tapping was required in 1.29% of them. The culture failure rate was 2.01%. The fetal loss rate after amniocentesis was 0.6%.There were no significant differences in the factors affecting fetal loss that included maternal age, gestational age, placental puncture, operator, number of tappings, myoma uteri and color of amniotic fluid. Pregnancy outcome of the fetus with normal chromosomes included fetal death (0.3%) and preterm delivery (5.1%).

Conclusion: Second trimester genetic amniocentesis at Khon Kaen University was a safe procedure in prenatal diagnosis of chromosomal abnormalities.

Keywords: Amniocentesis, Chromosomal abnormalities, Fetal loss, Prenatal diagnosis, Second trimester

Introduction

Amniocentesis is the most commonly performed invasive procedure for prenatal diagnosis of genetic diseases[1]. It is usually performed between 15 and 20 weeks of gestation[2]. This procedure was first introduced in the 1950s and was then used only for antenatal sex determination[3]. Since then, Steele and Breg cultured amniotic cells and analyzed their karyotypes in 1966[4]. Subsequently, amniocentesis has been done for diagnosis of a variety of disorders including chromosomal abnormality.

Amniocentesis for prenatal diagnosis of chromosomal abnormalities has been performed at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand since 1992. After that, the numbers of genetic amniocentesis procedures has been increasing because of the increasing numbers of elderly pregnant women and this institute is the tertiary referral center.

In Thailand, amniocentesis was performed in many institutions. There are many reports of their experiences[5-10]. The purpose of this study is to evaluate the results of second trimester genetic amniocentesis with special reference to fetal loss rate after the procedure in this institute.

Materials and Methods

From January 1, 1993 to December 31, 2006, a total of 2,825 cases of genetic amniocentesis were done on high-risk singleton pregnant women. Before amniocentesis, each patient had an ultrasound examination to confirm gestational age, fetal viability, placental location, amniotic fluid volume and anomalies. Genetic counseling had been provided for all pregnant women before undertaking the procedure.

Amniocentesis was performed under simultaneous free hand ultrasound guidance with a 20-guage spinal needle. Before starting the procedure, the operators cleaned the site of puncture by 70% ethanol solution. Initially, the first 1 ml. of amniotic fluid was discarded in order to avoid maternal cell contamination and then 1 ml per 1 week of gestational age of amniotic fluid was aspirated in the same syringe and sent for cell culture.

Fetal karyotyping was performed using Giemsa-Trypsin-G-banding at the Cytogenetics Unit, Department of Pathology, Srinagarind Hospital.

We collected patient demographic data, amniocentesis record forms, complications and pregnancy outcomes from medical records and questionnaires sent by mail. Questionnaires were mailed to 1,840 Srinagarind Hospital amniocentesis patients who had a valid mailing address. The mail response rate was 75.5% (1,390 out of 1,840 cases).

All data were calculated by percentage and analyzed using by Stata version 10 (STATA Corp, College Station, TX). This study was approved by the Human Research Ethics Committee of Khon Kaen University.

Results

A total of 2,825 amniocentesis procedures were performed during the study period. The number of genetic amniocenteses had been increased every year from 42 cases in 1993 to 406 cases in 2006.

The most common range of maternal age performed amniocentesis was 35-39 years old (66.3%). The most common range of gestational age performed amniocenteses was during 17-19 weeks (77.9%). 52.7% and 47.3% of amniocenteses were performed by staff and residents, respectively. Most women were referred from other hospitals (66.2%). The most common indication for amniocentesis was advanced maternal age (90.7%). Other indications were previous chromosomal abnormality (3.8%), previous fetal anomalies (2.1%), abnormal sonographic findings (1.5%) and others (1.9%) (Table 1).

Table 1. Indication for amniocentesis (N=2,825)

Indication	Number	Percent
Advanced maternal age	2,561	90.7
Previous chromosomal abnormality	107	3.8
Previous fetal anomalies	60	2.1
Abnormal sonographic findings	41	1.5
Recurrent pregnancy loss	15	0.5
Maternal anxiety	13	0.4
Family history of chromosomal abnormality	13	0.4
Positive biochemical Down syndrome screening	10	0.4
Previous fetal loss	5	0.2
Total	2,825	100.00

Table 2. Factors affecting fetal loss within 2 weeks after procedure (N=1,390)

Factors	No.	Fetal loss	p-value
1. Maternal age			
<35 years	161	2	0.28
>=35 years	1,229	7	
2. Gestational age			
<18 weeks.	219	0	0.37
>=18 weeks.	1,171	9	
3. Site of puncture			
Placental penetration	290	3	0.41
No	1,100	6	
4. Operator			
Staff	696	7	0.18
Resident	694	2	
5. Number of tapping			
1 tapping	1,371	8	0.12
>1 tapping	19	1	
6. Myoma			
Myoma	18	0	1.00
No	1,372	9	
7. Color of amniotic fluid			
Yellow	1,362	8	0.17
Other	28	1	

p-value < 0.05 is statistical difference.

It was found that the overall number of genetic amniocenteses for each occasion of pregnancy were those who had 2 procedures were 90 cases (3.2%) and 1 case (0.04%) had the procedure 3 times.

The abnormal chromosomal analysis's results were overall 3.1%. They consisted of numerical abnormalities in 2.2%, structural abnormalities in 0.5% and in the others category,0.4%. In numerical abnormalities, it was found that trisomy21 was present in 0.9%, trisomy18 in 0.4%, trisomy13 in 0.2% and others in 0.7% . In 107 cases with a previous history of chromosomal abnormalities, there were 3 cases or 2.8% with abnormal chromosomes.

Most amniotic fluid specimens were successfully obtained in the first attempt (98.68%). More than one puncture of the procedure was 1.29% and only one case or 0.03% in which the procedure was unable to be performed due to obesity.

Overall culture failure rate was 2.01% which included no metaphase (1.43%), bacterial contamination (0.43%) and no data (0.14%). A gestational age of less than 18 weeks was only the factor affecting culture failure that had statistical significance (p-value 0.002), while the color of amniotic fluid had no statistical significance.

From the questionnaire data (1,390 cases), fetal loss after amniocentesis within 2 weeks was 9 cases (0.6%). All of them had normal chromosomes. The other complications within 2 weeks were 1.3% amniotic fluid leakage, 1.2% maternal fever and 0.9%. vaginal bleeding.

Factors affecting fetal loss within 2 weeks are shown in Table 2. No statistical difference was found in all factors affecting fetal loss such as maternal age, gestational age, placental puncture, operator, number of tappings, leiomyoma and color of amniotic fluid.

Pregnancy outcomes after amniocentesis with normal chromosome profiles were full term pregnancy (94.6%), preterm delivery (5.1%) and fetal death after 2 weeks (0.3%).

The overall results of amniocentesis were compared with the reports from other institutes in Thailand as shown in Table 3.

Table 3. Comparison of genetic amniocentesis studies in Thailand

Institute	Chulalongkorn[5]	Songklanagarind[6]	Pramongkutklao[7]	Bhumibol[8]	Rajavithi[9]	Khon Kaen University
Period	June 1991-May 1992	January 1988-December 1997	October 1990-September 1996	January 1996-December 1999	January 1999-December 2002	January 1993-December 2006
Number (cases)	250	1,016	917	920	1,406	2,825
Most indication (%)	Elderly (84.8)	Elderly (97.9)	Elderly (86.5)	Elderly (92.2)	Elderly (94.1)	Elderly (90.7)
Trisomy21(%)	0.8	NA	0.89	0.43	0.92	0.9
Puncture failure (%)	NA	NA	NA	NA	NA	0.03
Multiple puncture (%)	4.1	NA	NA	3.48	3.66	1.29
Culture failure (%)	1.95	2.1	1.74	0.21	1.8	2.01
Fetal loss (%)	0	0.3	NA	0.21	1.6	0.6

NA = not applicable

Discussion

Genetic amniocentesis at Khon Kaen University was established in 1992. The numbers of genetic amniocentesis patients have been increasing every year. Advanced maternal age was the most common indication for prenatal diagnosis (90.7%), which is similar to other studies in Thailand[5-10]. The most common gestational age performed in this institute was 17-19 weeks. The prevalence of all chromosomal abnormalities in this institute was 3.1% and the prevalence of trisomy21 was 0.9%, which is similar when compared with other studies[5-10].

The culture failure rate in our study was 2.01%. This rate was comparable to other studies in Thailand[5-9]. The reported rate was lower than the western countries[1]. The difference may be due to the ability of the laboratory. It was found at the time of amniocentesis, only gestational age affected culture failure and this was in patients who were less than 18 weeks of gestational.This probably due to a small number of fetal cells in the amniotic fluid[1].

Several large collaborative observational studies have been undertaken to establish the safety of mid-trimester amniocentesis. The procedure-related fetal loss rate in these studies was in the range of 0.5-0.86%[10,11]. In our study, the total fetal loss rate within 2 weeks after procedure (0.6%) was comparable[2,5,7-9]. Odibo AO, et al[12], reported that total fetal loss rate was not significantly different from that observed in patients who did not have amniocentesis procedure. In this study it was not possible to demonstrate this difference due to the study was retrospective and lack a control group.

It has been reported that factors found to be associated with increased risk of fetal loss included needle gauges larger than 20-gauge, placental perforation, the presence of discolored amniotic fluid and more than two needle insertions at any given time[13] and performed by obstetrician-gynecologists when compared with perinatologists[14]. In this study, however, no statistical differences were found in factors affecting fetal loss such as maternal age, gestational age, placental puncture, operator, number of tapping, leiomyoma and color of amniotic fluid. Hankins GDV, et al[15], reported that discolored amniotic fluid during second trimester genetic amniocentesis, as an isolated finding, did not prognosticate a poor pregnancy outcome.

It has been previously reported that preterm labor rate after amniocentesis was 5.9-9.0%[6,16] which was not different from our study. Their fetal death after amniocentesis was 0.8-1.1% which was higher than reported in this study.

The limitation of this study was a retrospective study, which could have a recall bias, especially using data from questionnaires. Culture failure rate in our study was quite high due to a problem in culture system in early stage. Moreover, there was a small number of fetal loss, further study is supposed to be performed to evaluate the actual risk of fetal loss by univariate analysis.

A larger sample size was reported in this study than in previous studies in Thailand. The results from the present study could be useful in pre-procedure counseling for pregnant women who have an indication for genetic amniocentesis, in order to increase acceptance of this procedure[17-18].

Second trimester genetic amniocentesis at Khon Kaen University is a safe procedure for prenatal diagnosis of chromosomal abnormalities. The study showed fetal loss rate was only 0.6%.

Acknowledgements

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การเจาะถุงน้ำคร่ำเพื่อวินิจฉัยโรคทางพันธุกรรมในไตรมาสที่สอง:ประสบการณ์ 14 ปี ที่ มหาวิทยาลัยขอนแก่น

ถวัลย์วงค์ รัตนสิริ, รัตนา คำวิลัยศักดิ์, ธีระยุทธ เต็มธนะกิจไพศาล, สมาน เลืองวัฒนะวนิช, วิทูรย์ ประเสริฐเจริญสุข, ปิยะมาศ ศักดิ์ศิริวุฒโฒ, จำรัส วงศ์คำ

วัตถุประสงค์ *: เพื่อประเมินการเจาะถุงน้ำคร่ำเพื่อวินิจฉัยก่อนคลอดโรคทางพันธุกรรมในไตรมาสที่สองที่มหาวิทยาลัยขอนแก่น*

วัสดุและวิธีการ *: วิเคราะห์ข้อมูลที่ได้จากเวชระเบียนและแบบบันทึกการเจาะถุงน้ำคร่ำรวมทั้งหมด 2,825 ราย ระหว่าง 1 มกราคม 2536 ถึง 31 ธันวาคม 2549 ที่ รพ.ศรีนครินทร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น และแบบสอบถามที่ส่งทางไปรษณีย์เกี่ยวกับภาวะแทรกซ้อนจากการเจาะถุงน้ำคร่ำและผลของการตั้งครรภ์ ได้ส่งแบบสอบถามทางไปรษณีย์ โดยมี การตอบกลับแบบสอบถามร้อยละ 75.5*

ตัววัดที่สำคัญ *: อัตราการสูญเสียทารกในครรภ์หลังการเจาะถุงน้ำคร่ำภายในสองสัปดาห์*

ผลการศึกษา *: ข้อบ่งชี้ในการเจาะถุงน้ำคร่ำในไตรมาสที่สองที่พบมากที่สุดคือ มารดาที่อายุตั้งแต่ 35 ปีขึ้นไป (ร้อยละ 90.7) ผลของโครโมโซมที่ผิดปกติพบร้อยละ 3.1 ผลโครโมโซมผิดปกติที่พบมากที่สุดคือไตรโซมี 21 (ร้อยละ 0.91) มีเพียง 1 ราย (ร้อยละ 0.03) ที่ไม่สามารถทำการเจาะถุงน้ำคร่ำได้ พบว่าการเจาะถุงน้ำคร่ำโดยใช้เข็มแทงมากกว่า 1 ครั้งพบเพียงร้อยละ 1.29 พบอัตราความล้มเหลวในการเพาะเลี้ยงเซลล์จากน้ำคร่ำ ร้อยละ 2.01 อัตราการสูญเสียทารกในครรภ์หลังการเจาะถุงน้ำคร่ำภายในสองสัปดาห์พบ ร้อยละ 0.6 ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติในเรื่องปัจจัยที่ทำให้เกิดการสูญเสียทารกในครรภ์ ไม่ว่าจะเป็นอายุของสตรีตั้งครรภ์ อายุครรภ์ ตำแหน่งที่เจาะถุงน้ำคร่ำ ผู้เจาะ จำนวนครั้งของการเจาะ การมีเนื้องอกมดลูก หรือสีของน้ำคร่ำที่เจาะได้ ผลของการตั้งครรภ์หลังการเจาะถุงน้ำคร่ำของทารกในครรภ์ที่มีผลโครโมโซมปกติ พบว่ามีทารกตายในครรภ์ (ร้อยละ 0.3) และคลอดก่อน กำหนด (ร้อยละ 5.1)*

สรุป *: การเจาะถุงน้ำคร่ำเพื่อวินิจฉัยโรคทางพันธุกรรมในไตรมาสที่สองที่มหาวิทยาลัยขอนแก่น เป็นหัตถการที่ปลอดภัยในการวินิจฉัยก่อนคลอดเกี่ยวกับความผิดปกติของโครโมโซม*