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Assessment of Second Trimester Genetic Amniocentesis: A Review of 6 Years of Experience at Sanpatong Hospital, A Mid-level Secondary Hospital Setting

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ABSTRACT

OBJECTIVE This study aims to assess the indications, complications, and outcomes of second-trimester genetic amniocentesis performed at Sanpatong Hospital, Chiang Mai, Thailand.

METHODS A cross-sectional descriptive study analyzed data collected from high-risk pregnant women who underwent second-trimester genetic amniocentesis at Sanpatong Hospital between October 1st, 2016 and September 30th, 2022. The data include indications for the procedure, complications, and pregnancy outcomes.

RESULTS A study of 451 women with high-risk pregnancies who underwent amniocentesis found that the most common indications for second trimester genetic amniocentesis were advanced maternal age (49.4%) and a high-risk Quad test (49.4%). Abnormal chromosomes were detected in 3.1% of cases, with aneuploidy the most common type (2.1%), primarily trisomy 21 (1.3%). The overall aspiration success rate was 100%. The only complications related to the procedure were pelvic pain (0.6%) and placental hematoma (0.2%). There were no fetal losses within 30 days after amniocentesis. The culture failure rate was 1.1%. Pregnancy outcomes included preterm delivery (12.3%) and normal term delivery (87.7%).

CONCLUSIONS Performing second trimester genetic amniocentesis at Sanpatong Hospital, a mid-level secondary hospital, over a six-year period resulted in no fetal losses.

KEYWORDS amniocentesis, second trimester, genetic, chromosome abnormalities

INTRODUCTION

Amniocentesis is a prenatal diagnostic procedure that involves withdrawing amniotic fluid from the amniotic sac for cell culture (1). The resulting cells are then separated and analyzed for chromosomal abnormalities in the fetus. This procedure was first performed in the 1950s and was used to determine fetal sex (2). Amnionic cell culture and testing of fetal karyotypes was first successfully used in 1966 (3). Subsequently, amniocentesis became a standard procedure for diagnosing various chromosomal abnormalities.

The optimal gestational age for performing amniocentesis is between 15 and 20 weeks of pregnancy (4). Previous studies have, however, have reported complications associated with this procedure including abdominal cramping, pelvic pain, membrane leakage, infection, and fetal loss (5-8). Nevertheless, second-trimester genetic amniocentesis is considered safe when realtime

ultrasound guidance is employed for needle placement (8).

Despite inherent risks, amniocentesis has remained a crucial diagnotic tool for pregnant women at high risk of fetal chromosomal abnormalities. This includes women of advanced maternal age, a family history of chromosomal anomalies, and those with concerning results from prenatal ultrasound or high-risk Quad Tests (2).

Amniocentesis in Thailand is primarily performed in large hospitals, including provincial hospitals, Centers of Excellence hospitals, and medical university hospitals. However, Sanpatong Hospital, a mid-level secondary hospital, only began offering this procedure in 2016 to help reduce the workload and to alleviate the strain on resources at larger institutions. This initiative aimed to increase access to this service for high-risk pregnant women, while simulta-neously reducing their travel time and financial burden. Since the start of second-trimester genetic amniocentesis at Sanpatong Hospital, however, there has been no data regarding the safety of the procedure in the hospital or the prevalence of abnormal chromosomes.

The purpose of this study was to assess the indications, complications and outcomes of second-trimester genetic amniocentesis performed in Sanpatong Hospital.

METHODS

This study was approved by the Sanpatong Hospital Research Ethics Committee (approval number: SPT-REC 001/2566). The study analyzed data collected from high-risk pregnant women with fetal chromosomal abnormalities, including women of advanced maternal age (>35 years at the estimated due date), a family history of chromosomal anomalies, those with concerning results from prenatal ultrasound or high-risk Quad Tests (abnormal level of biochemical profile of alphafetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and inhibin A (inh A), which increase the risk of aneuploidy of trisomy 13, 18 and 21 to more than 1:250), between October 1st, 2016 and September 30th, 2022 who underwent a second-trimester genetic amniocentesis at the antenatal care unit of Sanpatong Hospital, Chiang Mai, Thailand.

The sample size for this study was calculated using the following formula:

$$n = \frac{N(Z\alpha/2)^2P(1-P)}{d^2(N-1) + (Z\alpha/2)^2P(1-P)}$$

where $Z_{\alpha/2}$ represents the 95% confidence level, P represents the prevalence of fetal chromosomal abnormalities (2.5%), N represents the total population size (451 cases), and d represents the desired level of precision (98%). The calculated sample size was 155 cases. As the population size was relatively small, the entire population was included in the study.

High-risk pregnant women were fully counseled on the benefits and risks of amniocentesis prior to the procedure. Informed consent was obtained for all procedures.

After counseling, a detailed ultrasound examination was performed using a Toshiba TUS-X100s ultrasound machine. This standard examination determined the number of fetuses, fetal viability, gestational age, amniotic fluid volume, and placental location.

The amniocentesis procedure followed standardized protocols. The abdomen was prepped with 10% povidone-iodine antiseptic, sterile drapes were applied and a 22-gauge spinal needle was inserted under realtime ultrasound guidance used a "two-person technique." One team member scanned the image while the other aspirated the amniotic fluid with three 10-cc syringes. The first syringe collected 1-2 cc, while the following syringes collected 8 cc each.

After the needle had been withdrawn, the women were shown the puncture site, the ultrasound image confirming the well-being of the fetus, and the measured amniotic fluid volume. After a 30-minute rest period, the women were discharged from the hospital if no complications had arisen. A follow-up appointment was scheduled one month later to discuss the results of the analysis with the patient and to monitor for potential complications. Additionally, the women were instructed to immediately seek medical attention at Sanpatong Hospital if any complications arose.

The collected amniotic fluid was subsequently sent to Bangkok Cytogenetics Center Co., Ltd. in Bangkok, Thailand for fetal karyotype analysis. When the cell culture was successful, the laboratory reported the fetal karyotype. If a cell culture

failed or if there was no cell growth, a QF-PCR test was performed by the Center and results were reported within a week.

This study collected data on demographic information, obstetric information, indications for amniocentesis, results of amniocentesis, complications, and pregnancy outcomes. The data are presented as counts, percentages, means, and standard deviations.

RESULTS

Our study identified 451 high-risk pregnant women who received second-trimester genetic amniocentesis at Sanpatong Hospital, Chiang Mai, Thailand, for various indications between October 1st, 2016, and September 30th, 2022. Of these women, the majority (51.3%) were aged 35-39 years. Notably, among mothers who underwent amniocentesis and had fetuses with chromosomal abnormalities, the highest proportion (35.7%) were in the 35-39 age group, followed by the 25-29 age group (28.6%). Ninety percent of the procedures were performed between 16 and 18 weeks of gestation (range 16-23 weeks) (Table 1).

Our study found the most common indication for amniocentesis was advanced maternal age, which has the same risk as high-risk serum screening (Quad test), accounting for 49.4% (223/451) of cases. Abnormal ultrasound findings were the second most common indication at 1.2% (5/451). Some cases had two indications for amniocentesis, including both advanced maternal age and high-risk serum screening (Quad test), accounting for 19.1% (86/451) of cases. The most common indication found to be associated with chromosomal abnormalities was high-risk serum screening (Quad test), accounting for 64.3% (9/14) of cases followed by advanced maternal age at 28.6% (4/14) and ultrasound abnormalities at 7.1% (1/14) (Table 2).

In our study the success rate of cell culture from amniocentesis was 98.9% (446/451). Five cases (1.3%) failed to culture cells, but all were found to have no chromosomal abnormalities of chromosomes 13, 18, 21, X, and Y by QF-PCR. The prevalence of fetal chromosomal abnormalities

Table 1. Maternal characteristics

Parameter	Amniocentesis		Abnormal chromosome	
	Number (n=451)	Percent	Number (n=14)	Percent
Maternal age (years)				
< 20	10	2.2	1	7.1
20-24	25	5.5	0	0.0
25-29	56	12.4	4	28.6
30-34	55	12.2	1	7.1
35-39	231	51.3	5	35.7
40-44	69	15.3	3	21.5
≥ 45	5	1.1	0	0.0
Gestational age (weeks)				
< 16	0	0.0	0	0.0
16-18	406	90.0	13	92.9
19-21	44	9.8	1	7.1
> 21	1	0.2	0	0.0

Table 2. Indication of second trimester genetic amniocentesis

Indications	Amniocentesis (n=451)	Percentage	Abnormal chromosome (n=14)	Percentage
Advanced maternal age	223	49.4	4	28.6
Positive serum screening	223	49.4	9	64.3
- Age < 35 years	137	30.4	5	35.7
- Age ≥ 35 years	86	19.1	4	28.6
Anomaly from ultrasound	5	1.2	1	7.1

Table 3. Assessment of clinical data

Indications	Abnormal chromosome (n=14)	Percentage
Culture result		
- Successful	446	98.9
- Failure	5	1.1
Chromosomal result		
- Normal	432	95.8
- Abnormal	14	3.1
- Failed culture result	5	1.1

was 3.1% (14/451) (Table 3).

Among the 14 cases of abnormal chromosomes, trisomy 21 was the most common, accounting for 1.3% (6/446). The remaining cases comprised various abnormalities, including translocation (1.0%, 4/446) and sex chromosomal abnormalities (0.4%, 2/446) (Table 4).

All complications were identified within the 30-day follow-up period following the procedure. Minor complications, including pelvic pain (0.6%) and placental hematoma (0.2%), were subsequently managed effectively with conservative treatment (Table 5). Notably, no miscarriages were attributed to the procedure within this 30-day period.

The pregnancy outcomes of 203 cases (45.5%) with normal fetal chromosomes were followed, including 25 cases (12.3%) of preterm delivery. Among the preterm deliveries, 2 cases (1%) were extremely preterm deliveries, 4 cases (2%) were very preterm, 3 cases (1.5%) were moderately preterm, 16 cases (8%) were late preterm, and the remaining 178 cases (87.7%) were normal term (Table 6).

DISCUSSION

Second-trimester genetic amniocentesis is a standard procedure that involves withdrawal of amniotic fluid from the amniotic sac for cell culture and diagnosis of fetal chromosome abnormality. Previously, amniocentesis was performed by a single operator using an ultrasound guide to insert a needle and then place an ultrasound probe to aspirate the amniotic fluid. However, in this study, a two-person technique was used, with realtime ultrasound guidance and amniotic fluid aspiration. This technique was introduced to assess the indications, complications, and outcomes of second-trimester genetic amniocentesis performed at Sanpatong Hospital between 2016 and 2022. The results provide valuable insights into the safety and efficacy of

Table 4. Assessment of the chromosomal results

Results	Chromosomal results (n=446)	Percentage
Normal chromosome	432	96.9
- 46 XX	212	47.5
- 46 XY	220	49.4
Abnormal chromosome	14	3.1
Numerical abnormalities		
- Trisomy 18	2	0.4
- Trisomy 21	6	1.3
Sex chromosome abnormalities		
- 45 X	2	0.4
Structural abnormalities		
- 46 XY, inv(9)(qh)	2	0.4
- 46 XY,qh+	1	0.3
- 46 XX,t(1;4)(p13;q12)	1	0.3

Table 5. Complication of procedure within 30 days (N=4)

Complication	Number	Details	Outcome	Percentage
Placental hematoma	1	Intra-procedure	Continued to term	0.2
Pelvic pain	1	1 day after procedure	Continued to term	0.2
Pelvic pain	2	3 days after procedure	Continued to term	0.4

Table 6. Assessment of the chromosomal results

Outcome	Number	Percentage
Preterm delivery	25	12.3
- Extremely preterm (GA < 28 weeks)	2	1.0
- Very preterm (GA 28-31 weeks)	4	2.0
- Moderately preterm (GA 32-33 weeks)	3	1.4
- Late preterm (GA 34-36 weeks)	16	7.9
Full term delivery	178	87.7

this procedure in a mid-level secondary hospital setting. Most of the women who underwent amniocentesis were aged 35-39 years, with the most common gestational age for the procedure being 16-18 weeks. This aligns with existing literature (8-10). Notably, following the implementation of the Quad test policy at Sanpatong Hospital, the study found a 30.4% increase in the number of women under 35 years of age who were identified as high-risk by Quad test and who subsequently underwent amniocentesis. Among this group, 3.7% (5/137) of the fetuses were found to have chromosome abnormalities. Advanced maternal age and high-risk serum screening results were the most common indications for amniocentesis, followed by abnormal ultrasound findings. These findings likely reflect both the Quad test policy and the concerns of high-risk pregnancy women regarding the potential risks of amniocentesis. These findings diverge from previous studies (8-10) which have reported advance maternal age was the most common indication, but in our studies suggests a change practice for high-risk serum screening was the most common indication.

Our study had a high success rate of cell culture (98.9%), with only 1.1% of cases failing to culture cells, lower than other studies (8-10). Notably, studies in Western countries have reported culture failure rates as low as 0.44% (12). Factors potentially impacting cell culture success include maternal blood contamination, bacterial contamination, insufficient fluid volume, discolored amniotic fluid, and contaminated culture media (13). While some authors have suggested an association between amniotic fluid culture failure and fetal chromosomal abnormalities, we were unable to confirm this relationship in our study. All cases with documented procedures that resulted in amniotic fluid culture failure in this study exhibited normal fetal chromosomes.

The prevalence of fetal chromosomal abnormalities in this study was 3.1%, with trisomy 21 identified as the most common aneuploidy (1.3%). This observed rate exceeds those reported in other studies (8-10), potentially reflecting Sanpatong Hospital's role as a referral center serving the southern region of Chiang Mai province.

This study found no miscarriages attributable to the procedure within 30 days. One percent of women experienced a post-procedural abortion, a rate consistent with existing literature (8-10). Minor complications occurred in a small fraction of cases (0.6% pelvic pain, 0.2% placental hematoma) and were effectively managed conservatively. Previous research has identified factors potential contributing to amniocentesis complications, including needle gauge > 20, placental penetration and more than two needle insertions (14). Placental hematoma has been reported in up to 1.3% of cases (15, 16).

A key strength of the study was that it was the first to report on second-trimester genetic amniocentesis performed in a mid-level secondary hospital. The study found that standardized protocols were used for counseling, ultrasound examination, and the amniocentesis procedure. Additionally, data were collected on various aspects of the procedure, including indications, complications, and pregnancy outcomes.

A limitation of this study was the ability to follow up on only 42.2% of pregnancy out-comes. This was due to the fact that Sanpatong Hospital serves primarily high-risk pregnancy women who undergo amniocentesis. When their test result is normal, these women return to their home hospitals for antenatal care and delivery. This could have resulted in inaccurate data for miscarriages, preterm birth, or term birth.

CONCLUSIONS

This study demonstrated that second trimester genetic amniocentesis performed at Sanpatong Hospital, a mid-level secondary hospital, over a six-year period resulted in no fetal losses.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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