Mid-trimester Genetic Amniocentesis: GATA Experience

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OBJECTIVE: To evaluate the data about amniocenteses those were performed in our department and to compare indications, procedure related losses and pregnancy outcomes.

STUDY DESIGN: The study was carried out at the Department of Obstetrics and Gynecology, Faculty of Medicine, Gülhane Military Medical Academy retrospectively. We studied amniocenteses for chromosomal analysis between 2002 and 2004. The data of the patients were obtained from their records.

RESULTS: The mean (±SD) gestational age at amniocentesis was 17.9±1.3 weeks (range 16-22 weeks). The most common primary indications for amniocentesis were advanced maternal age (37.0%) and positive screening test result (36.5%). In 1452 of the procedures (99.7%), amniotic fluid was obtained by a single puncture. A chromosomal abnormality was detected in 47 women (3.2%) who preferred termination of pregnancy. The total fetal loss rate was 0 in 1456 (0.0%) within two weeks following amniocentesis

CONCLUSION: Amniocentesis is a safe and reliable technique with low fetal loss rates in complicated and uncomplicated pregnancies in our study group.
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Key Words: Amniocenteses, Prenatal diagnosis, Outcome, Fetal loss

Mid-trimester genetic amniocentesis has been the gold standard for prenatal diagnosis. It is well known that fetal or maternal complications associated with amniocentesis depend on the unique patient and operator characteristics of each prenatal diagnosis center; thus, complication rates may vary among them. Danish collaborative study, being the only randomized controlled trial, reported the procedure related fetal loss rate as 1.0%.[1] This study was carried out among young low-risk women who did not represent most women undergoing amniocentesis in real practice. Our institution has been performing all the invasive diagnostic procedures for more than a decade as a reference center. The literature promoted us to analyze indications, procedure related fetal loss rate, results of cytogenetic analysis and clinical outcomes following genetic amniocenteses performed in our institution.

Materials and Methods

This retrospective study was carried out at the Department of Obstetrics and Gynecology, Faculty of Medicine, Gulhane Military Medical Academy, Ankara, Turkey. Amniocenteses for chromosomal analysis performed during the period from January 1, 2002 to June 31, 2004 were studied. The routine of our department during the study period was to offer all pregnant women a second trimester serum screening test including AFP, uE3 and BhCG (cut off>1/350) and an amniocentesis to women aged 35 or more.

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Women exposed to amniocentesis were identified by records from the Department of Medical Biology and Genetics where all chromosomal analyses were performed and registered. Details from the amniocentesis procedures including the women's age, indication for amniocentesis, procedure related gestational age, number of needle insertions, color of the amniotic fluid, karyotype result and pregnancy outcome were obtained from the patient records of our department. Patients with twin pregnancies or a prior invasive diagnostic procedure and without a delivery record in our institution were excluded. In cases where data were incomplete or found to be incorrectly recorded, the departments were contacted and further information was collected, if available. The files of all cases with abnormal outcome were reviewed in detail.

The fetal losses were classified as spontaneous abortion (loss) before 24 weeks' gestation and as either intrauterine death or neonatal death (death of a live-born baby in the first month) when >24 weeks.[2] They were further separated into those in which the fetus was known to have a major or potentially lethal abnormality or condition identified before the procedure or those without such a condition. Finally, the losses were divided into those within two weeks of the procedure or more than two weeks after the procedure. The procedure-related pregnancy loss rate was defined by subtracting the losses in pregnancies with known lethal conditions and those occurring more than two weeks after the procedure from total pregnancy losses. Delivery before 28 and 37 completed weeks were classified as immature and premature, respectively; thereafter as term.

All the procedures were performed by anyone of the first three authors, who have extensive experience with this procedure. Previous to the amniocentesis, an ultrasound examination by means of an high definition ultrasound apparatus (Prosound SSD 5500 Aloka, Tokyo, Japan) equipped with a

2.5-5.0 MHz curved linear transducer, was performed to assess fetal viability and number, gestational age, fetal anatomy and placental location. Next, a pocket of amniotic fluid was identified avoiding the fetus, placenta, and umbilical cord. The abdomen was then prepared with an antiseptic solution. Then, under continuous ultrasonic guidance, a 20-22 gauge spinal needle was inserted into the pocket of fluid. Efforts were made to avoid perforation of an anteriorly located placenta. When perforating the placenta the thinnest part was chosen, and the cord insertion was visualized in order to avoid punction. The first 1 to 2 mL of amniotic fluid was discarded, then approximately 20 mL of fluid was collected with a new injector. Local anesthetics, progesterone or antibiotics were not used. Patients with Rh incompatibility were given 300 microgram of anti-D immunoglobulin following the procedure. All women were instructed to report any bleeding, contraction or leakage of amniotic fluid following the procedure. From each sample, two parallel cultures in flasks, according to conventional methods, were set up and karyotypes were prepared from primary cultures, rather than from secondary passage. All karyotypes were examined by using Giemsa banding method. A minimum of 20 cells were counted and at least two karyotypes were prepared for each case. Prenatal diagnoses were available in 8-15 days.

Results

The study population consisted of 1456 women aged 18 to 45 years old. The mean (±SD) age of the women was 31.3±6.2 years with the majority in the range of 25-39 years (Table 1). The mean (±SD) gestational age at amniocentesis was 17.9±1.3 weeks (range 16-22 weeks). Ninety-seven percent of the procedures were in the range of 16-20 weeks. The distribution of procedures per gestational week was showed in Table 2. The most common primary indications for amniocentesis were advanced maternal age (37.0%) and positive screening test result (36.5%) Details of indications are listed in Table 3.

Table 1. Age Groups of the Study Population

Range	n	%
<35	918	63.0
15-19	20	1.4
20-24	250	17.2
25-29	320	22.0
30-34	328	22.5
>35	538	37.0
35-39	412	28.3
40-44	124	8.5
45-49	2	0.1
Total	1456	100.00*

*: figures were rounded

In 1452 of the procedures (99.7%), amniotic fluid was obtained by a single puncture, whereas two insertions were needed in four (0.3%) cases. Transplacental insertion was carried out in 54 cases (3.7%) with no influence on the fetal loss rate. In 10 cases (0.6%) the amniotic fluid had abnormal color. 18 cases (1.2%) reported leakage of amniotic fluid within two days. In none of these cases had the placenta been traversed.

Table 2. Distribution of Procedures per Gestational Age

Range	16	17	18	19	20	21	22	Total
Number	166	492	328	254	172	42	14	1456
Percent	11.4	33.8	22.5	17.4	11.8	2.9	0.1	100

A chromosomal abnormality was detected in 47 women representing a prevalence of 3.2%. The karyotypes were consisted of 22 trisomi 21. 21 trisomi 18, 3 a balanced fetal translocation, and one 45-46 deletion. All these 47 women preferred pregnancy termination.

Pregnancy outcomes of the cases are listed in Table 4. Sixty-nine women (4.7%) had an induced abortion; 22 due to major or multiple structural abnormalities, 35 due to chromosomal abnormalities, 12 due to both of these abnormalities. In the twelve women terminated due to structural abnormalities the karyotypes were consisted of trisomi 18. There was no spontaneous abortion. There were five intrauterine deaths and one neonatal death accounting for a total pregnancy loss of six (0.4%). Five cases of stillbirth had severe fetal growth restriction detected between 25th and 30th weeks and these losses were possibly not related to amniocentesis. In the remaining fetus, an amniocentesis was performed at the 16th week due to maternal anxiety and was resulted in 46XX normal constitutional karyotype. An emergency cesarean was performed at 27th weeks due to chorioamnionitis, placental decolman and acute fetal distress. The newborn was died at the third day of life due to neonatal infection. Although there were 13 weeks between the procedure time and the pregnancy loss, it was hard to classify it as a spontaneous complication. Of remaining preterm twenty fetuses, fourteen had a birth weight of greater than 2500 gram. Of 18 women reporting leakage of amniotic fluid two had a preterm delivery; sixteen had a term delivery.

Discussion

It is essential for women to know the risk of fetal loss and maternal complications associated with amniocentesis before the procedure. The total pregnancy loss rate, a combination of the procedure related loss and the background loss rate, is thought to be influenced by many factors including maternal age, fetal number, gestational age, the indications for the procedure, the operator skill, and the certain technical risk factors.[3-9] Thus, pregnancy outcomes following amniocentesis depend on the unique characteristics of each prenatal diagnosis center and figures, inevitably, may vary among the centers. It should be mentioned that the real problem is not different outcomes from different centers; but lacking of an agreed classification method for reporting of them.

Table 3. Indications for Amniocenteses

Primary Indication	n	%
Advanced maternal age (AMA: >35)	370	25.4
AMA+Screen positive	138	9.5
AMA+Abnormal sonographic finding	18	1.2
AMA+Previous fetus suspected to have chromosomal disorders	6	0.4
AMA+Previous child with Trizomy	4	0.3
AMA+Maternal translocation+Previous child with Trizomy	2	0.1
Subtotal	538	37.0
Positive screening test	532	36.5
Previous fetus suspected to have chromosomal disorders	162	11.1
Abnormal sonographic finding	134	9.2
Maternal anxiety	54	3.7
Previous child with Trizomy	32	2.2
Family history of chromosomal disorders	20	1.4
Fetal risk of X-linked disorders	4	0.3
Subtotal	918	63.0
Total	1456	100.0

Table 4. Pregnancy Outcomes

	n	%
Abortions (<24 week)	69	4.7
Induced	69	4.7
Major structural abnormality	22	1.5
Chromosomal disorder	35	2.4
Both	12	0.8
Spontaneous	0	0.0
Deliveries (>24 weeks)	1387	95.3
Immature (<28 weeks)	3	0.02
Premature (<37 weeks)	23	1.6
Mature (>37 weeks)	1361	93.5
Total	1456	100.0

Our amniocentesis loss rate in complicated and uncomplicated pregnancies was very low when the losses due to lethal conditions were excluded. The total fetal loss rate in our study was 0 in 1456 (0.0%) within two weeks following amniocentesis or until 24 weeks of gestation; 3 in 1456 (0.2%) until 28 weeks of gestation, and 6 in 1456 (0.4%) until 37 weeks or term of gestation. Any one of these figures is lower than the Danish collaborative study (1.0%) which is still the gold standard for the safety of amniocentesis,[1] or the new studies (0.7-0.8%) taking into consideration the pitfalls of interpretation.[10,11] Ongoing monitoring of these figures will be necessary to show whether this low loss rate is a transitory or a real feature.

In order to support a defined method to compare results from different units as well as pre-procedural counseling of patients, the procedure-related pregnancy loss rate of our study was reported according to the method suggested by Nanal et al (loss within two weeks with no known lethal abnormality).[2] As noted by them, we do acknowledge that some of the later losses in our study could still be procedure-related and should not be ignored, and also that some of losses soon after any procedure may have happened anyway. Roper et al. found that the cumulative fetal loss rate reached a peak at 3 weeks post-procedure and stabilized by the fifth week.[8] This aspect of fetal loss seems also to be important to determine the weeks after the procedure that still carries an increased risk.

There have been many contrasting reports on the factors to be associated with increased rates of fetal loss or on the incidence of other potential amniocentesis-related complications including pre-procedural three or more first trimester abortions, a second-trimester miscarriage or termination of pregnancy, bleeding in the current pregnancy, uterine tumors, elevated maternal serum alpha-fetoprotein levels; intra-procedural transplacental needle insertion, needle puncture of the fetus, discolored amniotic fluid; or post-procedural leakage of amniotic fluid, amnionitis, vaginal bleeding, placental abruption, placenta praevia, premature rupture of membranes, preterm delivery, respiratory distress syndrome, talipes equinovarus, and infant pneumonia.[12-24] Most of these studies have not included non-exposed controls. When the cases with predisposing factors are excluded, the increase in procedure-related fetal losses is statistically nonsignificant.[25] Cederholm et al recently investigated the effect of amniocentesis on the risk of bleeding, placental abruption, complications related to amniotic cavity and membranes, abnormal labour, operative deliveries and the impact of gestational length at the time of the procedure in the women, 35 to 49 years old, with single births exposed to amniocentesis (n: 21.748) or not exposed (n: 47.854).[26] They concluded that amniocentesis was not associated with important adverse outcomes such as abruption or placenta praevia. Minor asso-

ciations were found for other maternal complications when amniocentesis was performed before 15 weeks of gestation. In present study minimal leakage of amniotic fluid occurred in nine cases and resolved within several days. Although it is possible that some minor findings would probably be underdiagnosed or was not recorded, our present findings suggest that the overall incidence of antenal and neonatal complications is no greater than that expected for a general population.

In conclusion, amniocentesis is a safe and reliable technique with low fetal loss rates in complicated and uncomplicated pregnancies in our study group.

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