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The factors affecting the procedure duration during second trimester genetic amniocentesis: retrospective analysis

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Abstract

Objective: Genetic amniocentesis is a method of prenatal diagnosis which is still the most commonly performed procedure today. The aim of this retrospective observational study was to analyze the factors affecting the duration of second trimester genetic amniocentesis.

Methods: We evaluated the files of 117 patients in the study who undergone second trimester amniocentesis for genetic diagnosis. The duration that the amniocentesis needle was inside the uterine cavity and the duration of total procedure were both recorded and the correlation of these parameters with clinical and demographic characteristics was evaluated. For each patient, the indications for the procedure and clinical information were also registered.

Results: The mean±SD values of 117 patients for age and the weeks of gestation were 31.7±5.4 years and 18.4±1.7 weeks, respectively. The mean±SD values of the time the amniocentesis needle was inside the uterine cavity and the total procedure time were 85.3±59.3 and 118.7±79.5 seconds, respectively. While there was no difference among the operators in terms of the duration that the amniocentesis needle was inside the uterine cavity (p=0.079), the total procedure durations were statistically different (p=0.004). The procedure was significantly longer in patients with vaginal bleeding prior to amniocentesis than in patients without vaginal bleeding before the procedure (115.0 vs 74.8 s; p=0.030). Both the appearance of the needle tip on ultrasound and the difficulty degree of procedure felt by the operators were found to have a significant impact on the duration of the procedure (p values were 0.024 and 0.030, respectively).

Conclusion: The results of this study objectively show that second trimester genetic amniocentesis is a relatively short procedure. The difficulty degree of the procedure, the appearance degree of the needle tip on ultrasound and the presence of vaginal bleeding prior to the procedure affect the duration of the procedure. A larger scale prospective study is needed on the subject.

Key words: Amniocentesis, second trimester, prenatal diagnosis.

İkinci trimester genetik amniyosentezde işlem süresine etki eden faktörler

Amaç: Genetik amniyosentez günümüzde halen en sık yapılan invaziv prenatal tanı yöntemidir. Bu retrospektif gözlemsel çalışmanın amacı ikinci trimesterde genetik amaçlı yapılan amniyosentezde işlem süresine etki eden faktörleri ortaya koymaktır.

Yöntem: Çalışmada ikinci trimesterde genetik tanı amaçlı yapılan 117 amniyosentez vakasının dosyaları tarandı. Amniyosentez iğnesinin uterus içinde kaldığı süre ve total süre kaydedildi ve bu parametrelerin klinik ve demografik özelliklerle olan ilişkisi araştırıldı. Her hasta için işlem endikasyonu ve klinik bilgiler kaydedildi.

Bulgular: Toplam 117 hastanın yaş ve gebelik haftası ortalama±SD değerleri sırasıyla 31.7±5.4 ve 18.4±1.7'dir. Amniyosentez iğnesinin uterus içinde kaldığı süre ve total işlem sürelerinin ortalama±SD değerleri sırasıyla 85.3±59.3 ve 118.7±79.5 saniye olarak saptandı. İşlemi yapan doktorlar arasında iğnenin içerde kaldığı süre yönünden fark bulunmazken (p=0.079); total işlem süreleri doktorlar arasında istatistiksel olarak farklı bulundu (p=0.004). Amniyosentez öncesi dönemde vajinal kanaması olan hastalarda kanaması olmayan hastalara göre işlem anlamlı olarak daha uzun sürdü (115.0 vs 74.8 sn; p=0.030). İğne ucunun ultrasonda görülme derecesi ve işlemi yapan hekim tarafından işlemin hissedilen zorluk derecesi işlem süresi üzerinde etkili bulundu (p değerleri sırasıyla 0.024 ve 0.030).

Sonuç: Bu çalışmanın sonuçları, ikinci trimesterde genetik amaçlı yapılan amniyosentez işleminin kısa süreli bir işlem olduğunu objektif olarak göstermiştir. İşlemin zorluk derecesi, iğne ucunun ultrasonda görülme derecesi ve işlem öncesi vajinal kanamanın varlığı işlem süresini etkilemektedir. Bu konuda daha geniş kapsamlı prospektif çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Amniyosentez, ikinci trimester, prenatal tanı.

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Introduction

Amniocentesis is a procedure applied for more than 40 years.[1] Despite the improvements in fetal DNA analysis methods in maternal blood recently, second trimester genetic amniocentesis is still the most commonly used invasive prenatal diagnosis method today.[2,3]

Second trimester genetic amniocentesis is the procedure of aspiring amniotic fluid by the needle inserted through ultrasound guiding into uterus and amniotic cavity through abdominal anterior wall, and it is traditionally performed between 15 and 20 weeks of gestation.

Generally, the localizations of fetus and placenta are determined before amniocentesis. If placenta is on anterior wall, the needle is inserted through a location without placenta, or by piercing placenta. During the procedure, the localization of the needle is continuously monitored by ultrasound as displaying the whole needle. Thanks to the increase in the resolutions of ultrasound devices, moving amniocentesis needle forward under monitoring is rather faster and reliable today.

Amniocentesis is a procedure used frequently during daily practice, and a significant part of the daily workflow of physicians in some busy clinics may be occupied only for amniocentesis. Thus, presenting factors affecting procedure duration and minimizing the duration for the procedure become significant. Although it is done at a great number, there is still limited data today about the duration length of amniocentesis and the factors affecting the duration.[4-7] Therefore, the aim of this study is to reveal the factors affecting the duration length of amniocentesis performed for genetic purposes at second trimester.

Methods

The files of patients who undergone amniocentesis at Gynecology and Obstetrics Clinic of Çanakkale Onsekiz Mart University between October 2012 and June 2013 were scanned retrospectively. Before the procedure, the patients and their husbands were informed verbally and in written about the technique and possible complications of the procedure. Informed consents were received from the couples who accepted to have the procedure. A total of 117 patients who undergone amniocentesis at second trimester for prenatal genetic diagnostic purpose due to various indications and had at least 15 weeks and 0 days of singleton gestation were included to the study. The procedure indications were considered as high risk in triple test, high risk in double test, high risk in quadruple test, advanced maternal age (≥35), major anomaly or minor marker presence at ultrasound, history of baby with chromosomal anomaly in family and anxiety presence in family. Ages of patients included to the study were between 20 and 42. Patients who had multiple pregnancies, oligohydramnios presence, vaginal bleeding in the last two days before the procedure, those who took aspirin or heparin within the last 12 hours before the procedure or who had missing data in their files were excluded from the study.

Medical histories of all patients included to the study including age, last menstrual period, gravida, parity, and abortion numbers were received; their heights/weights were measured and body mass indexes (BMIs) were calculated. Clinical and demographic data such as maternal age, weeks of gestation, procedure indication, laparotomy history, vaginal bleeding presence before procedure, placental localization, and presence of transplacental transmission were recorded.

Amniocentesis Procedure

Amniocentesis procedure was conducted as specified below as a standard in each patient. First, weeks of gestation, placental localization and amniotic fluid amount of all patients were determined by ultrasound (Voluson 730 pro, GE Healthcare, Milwaukee, WI, USA). Before the procedure, routine fetal structural anomaly screening was carried out and weeks of gestation were confirmed by fetal biometry. Before beginning the procedure, polyvidone iodine was applied to lower abdominal quadrants. A sterile area was established on the procedure zone by sterile cover and the needle insertion point was determined through the guidance of ultrasound. For the procedure, 15 cm and 22-gauge spinal needle was used on each patient as a standard. Upon the contact of ultrasound probe with the skin, it was considered that "total procedure duration" began. After insertion point was determined with the guidance of continuous ultrasound image, the needle was inserted as needle was making an angle of 45 degree against the probe and it was moved to the cavity from the region where there is no fetal part or umbilical cord segment. Transplacental transmission

Table 1. Demographic and clinical data of patients included to the study (n=117).

Variable	Mean±SD	Median (quarters)
Age (year)	31,7±5.4	31.0 (28.3-36.0)
BMI* (kg/m²)	25.6±4.8	25.0 (22.0-28.3)
Weeks of gestation	18.4±1.7	18.1 (17.0-19.3)
Gravida (n)	2.2±1.2	2.0 (1.0-3.0)
Parity (n)	0.3±0.5	0.0 (0.0-0.8)
Abortion (n)	0.4±0.8	0.0 (0.0-1.0)
duration that needle stayed inside (sec)	85.3±59.3	60.0 (50.0-105.0)
Total procedure duration (sec)	118.7±79.5	88.5 (60.0-179.3)

***BMI:** body mass index

was avoided as much as possible. When the needle entered into the cavity, it was considered that the "duration that needle stayed inside" began. In order to decrease the risk of mentioned maternal cell contamination, 1 ml amniotic fluid was taken by a separate injector, and disposed. Then, 16-20 ml amniotic fluid was aspired from all patients. After this process, with the retraction of needle, it is considered that both durations are ended and these durations are recorded. These two durations described are recorded as amniocentesis data for each patient at our clinic. The procedure was carried out as standard technique by 5 different operators through following same rules. All patients were monitored as inpatient for two hours in terms of acute complications, and then they were discharged from the hospital after their fetal heart beats were checked again. Local anesthesia was applied on no patient during the procedure.

The physician carrying out the procedure subjectively evaluated the appearance degree of needle tip on ultrasound as poor, average and good, and also the difficulty of the procedure as very easy, easy, average, hard, and very hard.

Statistical Analysis

The data of the study was analyzed by using IBM SPSS 20 (SPSS Inc., Chicago, IL, USA). The consistency of variables with normal distribution was analyzed by visual (histogram and probability graphs) and analytic (Kolmogorov-Smirnov test) methods. Mann-Whitney U test was used for the comparison of two independent groups in terms of numerical variables while Kruskal-Wallis test was used for the comparison of three or more independent groups. The paired comparisons were done by using Mann-Whitney U test, and they were analyzed by using Bonferroni correction. Arithmetic mean±SD and median (first and third quarter values) were given as descriptive statistics. $P<0.05$ was considered as statistically significant.

Results

Mean±SD values for age and weeks of gestation of totally 117 patients were 31.7±5.4 and 18.4±1.7, respectively. Mean±SD values for the duration that amniocentesis needle stayed within uterine and total procedure duration were found as 85.3±59.3 and 118.7±79.5 seconds, respectively. Demographic and clinical data of the patients included to the study were given in **Table 1**, and the indications of amniocentesis procedure were given in **Table 2.**

While there was no difference among the physicians for the procedure duration that the needle stayed inside (p=0.079), there was statistically significant difference for total procedure durations (p=0.004).

Table 2. The indications of genetic amniocentesis (n=117).

Indication	n	%
High risk in triple test	33	28.2
High risk in double test	28	23.9
High risk in quadruple test	25	21.4
Advanced maternal age (≥35)	12	10.3
Anomaly presence at USG*	12	10.3
History of baby with chromosomal anomaly	6	5.1
Anxiety presence in family	1	0.08

*Minor and major anomalies observed during ultrasound were included.

The procedure took long in patients who had vaginal bleeding before amniocentesis compared to those without vaginal bleeding (115.0 vs 74.8 sec; p=0.030). The appearance degree of needle tip on ultrasound and the difficulty level during the procedure that the physician had was found to have impact on the procedure duration (p values were 0.024 and 0.03, respectively). In paired comparisons conducted by Bonferroni correction, the appearance degree of needle tip on ultrasound being "poor" or "good" was found to have impact on the procedure duration (p=0.004). Similarly, in paired comparisons conducted by Bonferroni correction, there was difference in terms of the procedure duration between the "easy" and "average" groups for the difficulty level during the procedure that the physician had being "easy" or "average" (p=0.002).

Patient age below or above 35, and BMI below or above 30 kg/m^2, presence of laparotomy history, placental localization and transplacental transmission of needle had no impact on the duration that needle stayed within uterine (p>0.05) (**Table 3**).

On the other hand, no correlation was found between age, height, weight, BMI, weeks of gestation, gravida, parity and abortion number with the duration that needle stayed within uterine (p>0.05). No complication was observed in any case during procedures, except temporal fetal bradycardia which lasted only for 1-2 minutes in one case.

Discussion

The results of this study objectively showed that the amniocentesis is a short procedure which lasts for 1.5-

Table 3. Comparison of durations that needle stayed within uterine, according to clinical and demographic characteristics (n=117).

	The duration that needle stayed within uterine (sec)		
	Mean±SD	Median (quarters)	P*
Age (year)			
<35	86.1±108.8	60.0 (50.0-83.0)	0.706
≥35	94.0±72.6	64.0 (50.0-120.0)	
BMI† (kg/m^2)			
<35	79.7±54.9	65.0 (50.0-90.0)	0.822
≥35	80.8±54.9	68.0 (51.0-92.5)	
Laparotomy history			
Yes	96.6±71.0	75.0 (50.0-122.5)	0.317
N/A	67.5±33.7	60.0 (50.0-77.5)	
Placental localization			
Anterior	75.0±35.5	65.0 (51.3-90.0)	0.457
Other	84.1±66.7	69.0 (50.0-90.0)	
Transplacental transmission of the needle			
Yes	78.1±58.4	60.0 (50.0-88.3)	0.738
N/A	80.9±53.0	64.5 (53.5-90.0)	
The presence of vaginal bleeding before procedure			
Yes	115.0±99.7	75.5 (52.5-210.0)	0.030
N/A	74.8±43.4	61.0 (50.0-90.0)	
The appearance degree of needle tip			
Poor	175.0±106.1	175.0 (100.0-250.0)	0.024**
Average	106.1±79.9	90.0 (50.0-130.0)	
Good	68.9±36.6	60.0 (50.0-80.0)	
The difficulty level of the procedure‡			
Very easy	37.5±10.6	43.0 (30.0-63.0)	0.003#
Easy	66.8±33.5	60.0 (50.0-80.0)	
Average	117.5±78.3	90.0 (60.0-157.0)	
Hard	175.0±106.1	175.0 (100.0-250.0	

*Comparisons were performed by using Mann-Whitney U test and Kruskal-Wallis test. †BMI: body mass index **By conducting Bonferroni correction, statistically significant difference in paired comparisons were determined between "poor-good" groups (P=0,004). ‡The difficulty level of the procedure was assessed by the physician who carried out the procedure, and there was no patient in "very hard" group. #The statistically significant difference in paired comparison performed by Bonferroni correction was found between "easy" and "average" evaluations (p= 0.002).

2 minutes. This study also detected that the parameters such as the appearance degree of needle tip on ultrasound during amniocentesis procedure, the difficulty level of the procedure that physician had and the presence of vaginal bleeding before the procedure had an impact on the duration that needle stayed within the uterine during amniocentesis. Maternal age, BMI, laparotomy history, placental localization and transplacental transmission of the needle did not affect statistically the procedure duration.

Amniocentesis is known as a short procedure. In the prospective study performed on 316 amniocentesis patients, Tchirikov et al.[7] used 29-gauge atraumatic needle for the procedure. The authors reported that median value of the procedure duration as 4.0 minutes (quarters: 4.0-5.0). In our study, the median values for the duration that the needle stayed within the uterine and total procedure durations were 1 and 1.5 minutes, respectively (**Table 1**). Both durations are quite shorter than those reported in the study of Tchirikov et al. The reason for the duration difference of the studies may be caused by the diameter differences of needles used in the studies. We used 22-gauge (0.7 mm) needle in our study, but 29-gauge (0.34 mm) needle used by Tchirikov et al. which is thinner than our needle may cause the procedure duration to extend. Another possible reason is the possibility that procedure duration has not been defined similarly, because authors did not stated clearly the beginning and ending criteria for the procedure duration. Consequently, amniocentesis takes relatively short time and needles with small diameter may extend the procedure duration.

In another study[4] including 123 patients who undergone early amniocentesis (at 10-13 weeks of gestation, mean weeks are 12.3), mean amniocentesis period was reported as 4.02 minutes (95% confidence interval; range: 3 minutes and 36 seconds - 4 minutes and 18 seconds). On the other hand, total procedure duration in our study was found as 2 minutes (118 seconds). The duration found in this research was approximately two times higher than the duration found in our study. This difference between the durations might be caused by the fulfillment of amniocenteses at early weeks of gestation. Because the uterine volume is smaller during early weeks of gestation and the procedure might take long when the needle entered into the cavity. Similarly, total procedure duration might be defined differently in this study, and cause to find different procedure durations. In our study, there was no correlation between the weeks of gestation and procedure duration; however, the procedure duration might be found longer since the weeks of gestation is smaller at early amniocentesis.

In the study performed in 2005 and included 50 amniocentesis cases,[5] the impact of 4D ultrasound on amniocentesis procedure was analyzed. The authors reported that they used 2D and then 4D ultrasound during the procedure, and that the mean value of total procedure duration was 1.5±0.7 minutes. In our study, mean total procedure duration was 118.7±79.5. Our result seems to be consistent with the result above. Similarly, in another study comparing the impact of 4D and 2D ultrasound procedures,[6] mean duration of 100 procedures performed by 2D ultrasound was reported as 25±5.5 seconds. This short duration may be caused by the different definition of the duration recorded. Studies discussed above and our results showed that second trimester genetic amniocentesis is a short-duration prenatal diagnostic method even though there are seconds of differences.

During the design phase of the study, we aimed to reveal the impacts of possible factors affecting amniocentesis procedure durations on both "the duration that the needle stayed inside" and "the total procedure duration". However, as we found total procedure duration statistically different among physicians who conducted the procedure (p=0.004), we did not carry out more analysis to see the impact of possible factors. Since the durations that the needle stayed inside were similar for all physicians (p=0.079), all analyses were performed for the durations that the needle stayed inside.

It is difficult to compare our results with others since there are very limited data in the literature for the factors affecting the duration amniocentesis procedure. In our study, the duration of the procedure was found to be longer when the needle tip was hardly seen on ultrasound or the procedure was difficult for the physician (p values were 0.024 and 0.030, respectively). It may be an expected condition that both cases extend the duration. However, the presence of vaginal bleeding (except last two days) before the procedure caused the procedure to last long (p=0.030). In order to compare this result, we could not access to the previous studies. On the other hand, patient age below or above 35, and BMI below or above 30 kg/m^2 did not affect the procedure duration (p values were 0.706 and 0.822,

respectively). The procedure duration might be expected to last long in patients with higher BMI values; however, it had no impact on the duration. This result showed that amniocentesis was a short-duration procedure even on overweight patients.

Our study has some limitations. The most important limitation is the lack of perinatal results. The possible relationship between perinatal morbidity and mortality with amniocentesis duration (i.e. long procedure duration may be together with increased pregnancy loss) might reinforce our study. In order to put forth the factors affecting the duration of amniocentesis procedure, it is undoubtedly be the best to design a larger scale of prospective study. Being retrospective and including rather less patients may be considered as the other limitations. The strength of our study is to be the first putting forth the factors affecting amniocentesis durations according to the literature.

Conclusion

The results of this study objectively show that genetic amniocentesis performed in second trimester is a relatively short procedure. The degree of difficulty of the procedure that physician had, the appearance degree of the needle tip on ultrasound, and the presence of vaginal bleeding prior to the procedure affect the duration of the procedure. A larger scale prospective study is needed to put forth the factors affecting the duration of amniocentesis procedure.

Conflicts of Interest: No conflicts declared.

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