The effect of diagnostic amniocentesis and its complications on early spontaneous abortion

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Abstract

Introduction: The occurrence of early abortion after amniocentesis is a serious problem in the fields of obstetrics and gynecology, and it is always important to discover the factors influencing this phenomenon. The incidence rate has been reported in different studies, even up to about 10%. So far, no studies have been conducted in Iran on the effect of amniocentesis and related complications on early abortion. The aim of this study was to determine the effects of amniocentesis and relevant complications on the incidence of early abortion in pregnant women undergoing amniocentesis.

Methods: This cohort study was conducted between March 2014 and March 2016 on pregnant candidates for amniocentesis referred to the perinatology clinic at Ommol-Banin Hospital, Mashhad, Iran. Amniocentesis was performed for all patients with about 20-30cc in the same manner by a perinatologist. Maternal blood group, causes of amniocentesis, amniotic fluid profile (liquid color), status of inserting the needle through the placenta during amniocentesis, amniotic fluid leakage, and bleeding after amniocentesis were considered as exposure factors, and spontaneous abortion after amniocentesis until the end of the 20th week of pregnancy was taken as a consequence. Data were analyzed using IBM-SPSS version 20 via t-test and chi-square. Relative risk (RR) was calculated to determine the causal relationship of exposure with the consequences of spontaneous abortion during the first week after amniocentesis.

Results: This study was performed on 1000 pregnant women with mean age of 33.4±6.0 years (minimum 16, maximum 48 years). The incidence rate of spontaneous abortion after amniocentesis was obtained 1%. There was no association among causes of amniocentesis, maternal blood group, maternal underlying diseases, history of diseases associated with pregnancy, and spontaneous abortion. Based on the chi-square test, a significant statistical relationship was found between amniotic fluid leakage and spontaneous abortion (RR=15.37, p=0.001). There was also a significant statistical relationship between bleeding after amniocentesis and spontaneous abortion; so that in patients with more bleeding, spontaneous abortion was more prevalent (RR=6.83, P=0.001).

Conclusion: According to the results, it seems that amniotic fluid leakage and bleeding after amniocentesis should be considered as two serious complications of amniocentesis, which can cause the incidence of spontaneous abortion in pregnant patients undergoing amniocentesis.

Keywords: Abortion, Amniocentesis, Complications, Pregnancy

1. Introduction

Amniocentesis means the extraction of amniotic fluid through a mother’s abdominal walls and is the most commonly used method for detecting chromosomal abnormalities (1). This procedure is usually performed between the 15th and 20th week of pregnancy, and early measures can lead to less success, increased unsuccessful cell culture, higher risk, and fatal complications (1, 2). The occurrence of early abortion after amniocentesis is a serious problem in the fields of obstetrics and gynecology, and it is always important to discover the factors influencing this
phenomenon (3, 4). The incidence rate has been reported in different studies, even up to about 10% (2-6). Amniocentesis is applicable for diagnostic and therapeutic measures, and the most diagnostic indications include prenatal genetic study, evaluation of fetal lung maturity, and assessment of fetal infection, anemia, determining the type of platelets or blood and neural tube defects (7-9). According to studies, bloody amniotic fluid occurs in less than 1% of amniocenteses, and the increased embryonic loss after bloody fluid has been reported in some studies; also, green or brown amniotic fluid has been associated with a higher rate of abortion in some studies (3, 9-11). In addition, other complications also have been shown, such as uterine contractions, temporary bleeding, loss of amniotic fluid, rupture of membrane, direct and indirect fetal damages as well as infection, which can all lead to abortion (12-14). However, no Iranian study has been conducted to investigate the effect of amniocentesis and related complications on early abortion with high statistical sample size; therefore, little information is available about this in Iran. By achieving a cause–effect relationship based on the results of the present study among the risk factors of early abortion after amniocentesis, the incidence rate of these adverse consequences can be reduced in the future by taking extra precautions. The aim of this study was to evaluate the effect of amniocentesis and its related complications on the incidence rate of early abortion in pregnant women undergoing amniocentesis.

2. Material and Methods
2.1. Research design and setting
This cohort study was conducted on pregnant patient candidates for amniocentesis referred to the perinatology clinic at Ommol-Banin Hospital, Mashhad, Iran, between March 2014 and March 2016.

2.2. Sampling
Considering alpha = 5%, beta = 20%, and P = 10%, the sample size was an estimated 1000 patients with the sample size formula based on prevalence of disease (14). All patients who were referred to our clinic during the study period were enrolled, and we used census method for sample selection.

2.3. Selection criteria
The inclusion criteria were 1) patients referred by a gynecologist due to abnormal biochemical or ultrasound markers detected during pregnancy; 2) history of anomaly in previous pregnancies or relatives such as Down’s syndrome; 3) women having blood relations with their husbands. The exclusion criteria were missing in the follow-up period.

2.4. Data collection
First, patients’ initial information was recorded such as age, gestational age based on the first day of the last menstrual period (LMP), body mass index (BMI), mother’s blood group, underlying diseases and medical records in previous pregnancies, and referral indication for amniocentesis. Then an ultrasound was performed by the same experienced sonographer to determine fetus’s living status, gestational age, the location of placenta, and the amount of amniotic fluid and to select the perfect spot for amniocentesis needle insertion. Before performing amniocentesis, the procedure and possible complications were explained to all patients plainly. Every step of amniocentesis was performed by an experienced perinatologist. First, the abdominal wall was disinfected. In an ultrasound-guided procedure, a needle number 23, manufactured by Dr.J (a Chinese company), was inserted into the skin of the mother’s abdominal wall through the gestational sac to take 20-30 ml of amniotic fluid approximately. In cases that mothers visited the clinic after a gestational age of 16 weeks and 3 days, an extra 10 cc of amniotic fluid was taken for an immediate test due to the time limitation on the abortion license. The patients were requested to visit the clinic in the presence of uterine cramps, hemorrhages, vaginal discharge, or fever signs during the first days after amniocentesis. Amniotic fluid color (bloody, transparent, green, or brown) and passage of the needle through placenta were also registered.

2.5. Exposure and Outcome
In this study, the factors of exposure included mothers’ blood group, reason for amniocentesis, specifications of amniotic fluid (color), needle passing status through the placental in amniocentesis, amniotic fluid leakage, and spotting after amniocentesis. Factors of outcome were considered miscarriage after amniocentesis.

2.6. Follow-up
Patients were under weekly follow-ups on the phone until the 20th pregnancy week for the incidences of amniotic fluid leakage, spotting, and abortion.
2.7. Research ethics
The procedure of amniocentesis and possible complications were explained to patients before conducting the study. All the patients filled out the consent form, indicating that they participated in the research plan consciously. This research plan was approved by the Ethics Committee of Mashhad University of Medical Sciences.

2.8. Statistical analyses
IBM® SPSS® Statistics version 20 (IBM® Corp., Armonk, NY, USA) was used for data analysis. The normality of data was first determined with the Kolmogorov-Smirnov test. If data were distributed normally, the t-test was used to determine the relationship between the mean of a quantitative variables in two groups with positive and negative outcomes. The U-Mann-Whitney test was conducted for non-normally distributed data. The chi-squared test was employed to determine the relationship between qualitative variables with positive and negative outcomes. Given the fact that this was a cohort study, the relative risk (RR) was calculated in order to determine the cause–effect relationship between exposures and outcomes of miscarriage during one week after amniocentesis. If RR was equal to 1, the exposure would not be related to the outcome. If it was greater or smaller than 1, it would be related to the final outcome.

3. Results
3.1. Baseline characteristics
During the study, 2213 pregnant women, who were candidates for amniocentesis, were referred to our clinic among whom 1032 patients were included. Later on, 32 of them were excluded from the study due to missing the follow-up. Finally, the study was carried out on 1000 pregnant women with the average age of 33.4 ± 6.0 years (minimum of 16 and maximum of 48 years) and with the average BMI of 26.1 ± 4.1 kg/m².

3.2. Incidence of spontaneous abortion
All patients underwent amniocentesis, and the follow-up after amniocentesis revealed that the total incidence of spontaneous abortion after amniocentesis was 1%. Based on the outcome (whether spontaneous abortion occurred during the following amniocentesis or not), the pregnant women were placed into two groups: the group with spontaneous abortion (10 pregnant women) and the group without it (990 pregnant women). There were no statistically significant differences between the groups with respect to the quantitative data of age, age of pregnancy, and average BMI (Table 1).

| Variable, unit                        | Spontaneous abortion (n=10) | No spontaneous abortion (n=990) | p-value |
|--------------------------------------|-----------------------------|---------------------------------|---------|
| Average age of pregnant women (years)| 33.47 ± 6.12                | 32.12 ± 6.28                    | 0.401   |
| Average age of pregnancy (weeks)     | 16.35 ± 3.31                | 15.81 ± 4.19                    | 0.339   |
| BMI (kg/m²)                          | 26.17 ± 4.34                | 25.83 ± 5.28                    | 0.721   |
| Amniocentesis indications, n (%)     |                             |                                 |         |
| Disorders detected in screening by ultrasound | 2 (20)                     | 43 (4.5)                        | 0.810   |
| Disorders detected in screening by biochemical markers | 5 (50)                     | 570 (58)                        |         |
| Other causes                         | 3 (30)                      | 337 (38.1)                      |         |
| Blood groups of pregnant women, n (%)|                             |                                 |         |
| A                                    | 4 (40)                      | 338 (34.1)                      | 0.394   |
| AB                                   | 1 (10)                      | 119 (12.0)                      |         |
| O                                    | 3 (30)                      | 293 (29.5)                      |         |
| B                                    | 2 (20)                      | 240 (24.2)                      |         |
| History of diseases in the pregnant women, n (%)| |                    |         |
| Diabetes                             | 1 (10)                      | 60 (6.0)                        | 0.500   |
| Preeclampsia                         | 1 (10)                      | 45 (4.5)                        |         |

3.3. Background status of pregnant women and spontaneous abortion
Results indicated no relationship among the reasons for performing amniocentesis, blood groups, background diseases, history of diseases related to pregnancy, and spontaneous abortion (Table 1).
3.4. Fetal screening and spontaneous abortion

Based on results of this study, there was no statistically significant relationship among disorders detected in screening by ultrasound or by biochemical markers and spontaneous abortion following amniocentesis (Table 1).

3.5. Characteristics of the amniotic fluid and spontaneous abortion

Amniocentesis was performed in every patient on the first attempt. In 94 patients (9.4%), the needle passed through the placenta during amniocentesis; in 42 patients (4.2%), the amniotic fluid drawn out of the mother’s abdomen was bloody. There were no statistically significant relationship between passing the needle through the placenta or the color of the amniotic fluid and spontaneous abortion (Table 2).

Table 2. Comparison of the groups with and without spontaneous abortions following amniocentesis based on features of amniocentesis and on its complications.

| Variable                              | Spontaneous abortion, \(n = 10\) | No spontaneous abortions, \(n = 990\) | \(p\)-value |
|---------------------------------------|----------------------------------|-------------------------------------|------------|
| Passage of needle through the placenta, number (%) | 0 (0)                            | 49 (4.9)                            | 0.999      |
| Color of the amniotic fluid, \(n\) (%) | Bloody                           | 1 (10)                              | 0.201      |
|                                       | Opaque or brown                  | 0 (0)                               | 0.999      |
| Other complications following amniocentesis | Leakage of amniotic fluid       | 2 (20)                              | 0.001      |
|                                       | Spotting                         | 1 (10)                              | 0.031      |

3.6. Short-term complications following amniocentesis

Leakage of amniotic fluid was observed in 16 patients (1.6%). In all such cases, the leakage started immediately after amniocentesis up to 24 hours after and stopped during the one-week follow-up. Moreover, spotting was also observed in 16 patients (1.6%) following amniocentesis. It started from 24 to 72 hours after amniocentesis and stopped during the one-week follow-up. There were two statistically significant relationships following amniocentesis between leakage of amniotic fluid and spontaneous abortion (RR = 15.37, \(p = 0.001\)). Moreover, a significant relationship was observed between spotting and spontaneous abortion (\(p = 0.001\), RR=6.83) (Table 2).

4. Discussion

Amniocentesis is a procedure in which amniotic fluid is extracted through the amniotic sac and is the most common method to detect genetic disorders of the fetus (2, 3, 5). If the procedure is performed early, it would have a lower success rate, and the cell culture would be unsuccessful. It also causes several fetal complications and risks (2, 5). In the present study, amniocentesis was performed on pregnant women with gestational age of less than 20 weeks. In cases in which the visit was after the gestational age of 16 weeks and 3 days, due to the time limit allowed for abortion, 10 ml extra of amniotic fluid was taken for immediate testing, and patients were followed-up weekly until the end of the 20th week of pregnancy. In this study, the relationship between the incidence of abortion following amniocentesis and the passage of needle through the placenta during amniocentesis was examined, for which statistical analysis showed no significant relationship. In spontaneous abortion cases, needles had not passed the placenta, while needles had passed the placenta in 4.9% of the cases without spontaneous abortion, which might be due to the position of placenta. However, Anderson (1989) stated that the position of placenta has no effect on the incidence of spontaneous abortion (15). Giorlando (1994) examined the amniocenteses that were performed through passing the placenta. In 1487 cases, the needle had passed anterior placenta; in 3077 cases, the needle had directly entered the amniotic fluid without passing the placenta. Patients were followed-up for 30 days after the amniocentesis (16). Two cases of spontaneous abortion were reported in the group with passage of the needle through placenta, and five cases of spontaneous abortion were reported in cases without passage of the needle through placenta. It was suggested that the risk of spontaneous abortion was similar in both groups. Martin (1997) reported no difference in risk of spontaneous abortion in anterior or posterior placenta cases and suggested that passage of the needle does not increase the risk of spontaneous abortion (17). In a study by Bombard (1995), placenta was anterior in 518 cases, and the needle had passed through the placenta in 306 amniocentesis cases. Spontaneous abortion following passage of the needle through placenta occurred within an average of 26 days after amniocentesis. The rate of fetal loss was similar in both groups and equal to 1.9% (18). Crane (1984) and Kang (2006) reported increased incidence of spontaneous abortion in cases where the needle had passed through the placenta (11, 19). In the present study, we investigated the relationship between the incidence of spontaneous abortion following amniocentesis and the amniotic fluid being bloody, non-clear, or brown. The amniotic fluid was bloody in 10% in the spontaneous abortion cases and in 3.2% of the cases without spontaneous abortion. The
statistical analysis showed increased incidence of spontaneous abortion in cases with bloody amniotic fluid. No case of brown or non-clear amniotic fluid was observed in the spontaneous abortion cases. Kappel (1987) reported an increased spontaneous abortion risk when the amniotic fluid is bloody. According to Taber (1986), non-clear amniotic fluid is associated with an increased risk of spontaneous abortion (20, 21). Hess (1986) examined the effect of abnormal color of amniotic fluid obtained from amniocentesis in 7018 subjects. The amniotic fluid color was abnormal in 1.4% of the cases, which increased the incidence of spontaneous abortion by 7% compared with the control group (22). In this study, we examined the relationship between the incidence of spotting and leakage of amniotic fluid following amniocentesis and the incidence of spontaneous abortion. In 22.2% of the cases with spontaneous abortion and in 1.2% of the cases without spontaneous abortion, amniotic fluid leakage occurred within the first 10 days after amniocentesis. Statistical analysis showed that leakage of amniotic fluid following amniocentesis increases the risk of spontaneous abortion. In 16.7% of the cases with spontaneous abortion and in 1.3% of the cases without spontaneous abortion, spotting occurred within the first 10 days after the amniocentesis. Statistical analysis showed that spotting following amniocentesis increases the risk of spontaneous abortion. Anderson (1989) and Koralo (2012) reported that history of bleeding in pregnancy is associated with an increased risk of spontaneous abortion following amniocentesis (15, 23). Maternal age and gestational age were other unrelated variables in our study, while a study conducted in 2011 showed that both variables are involved in spontaneous abortion before the gestational age of 24-28 weeks and the maternal age of 41 years. In the present study, maternal age, underlying and pregnancy-related disease, blood type, and body mass index were not related to spontaneous abortion following amniocentesis. As mentioned earlier, the only two factors of amniotic fluid leakage and spotting were causes of spontaneous abortion following amniocentesis. Amniotic fluid leakage was observed in 1.6% of the cases. In all patients, amniotic fluid leakage started after 24 hours after amniocentesis and ended within a week of follow-ups. Spotting was observed in 1.6% of cases following amniocentesis and between 24 and 72 hours after amniocentesis. It discontinued in all patients during the one-week follow-up. A statistically significant relationship was found between amniotic fluid leakage and spotting following amniocentesis and spontaneous abortion. The incidence of amniotic fluid leakage and spotting was higher in patients with spontaneous abortion. One of the major limitations of this study is in regards to its one center study.

5. Conclusions
According to the results, it seems that maternal baseline characteristics such as age, age of pregnancy, BMI, indication of amniocentesis, blood group, and disease history might not have a relationship with spontaneous abortion after amniocentesis. However, among the post-amniocentesis complications, leakage of amniotic fluid and spotting were two important factors that have been affecting spontaneous abortion. By the way, more observational studies are needed for confirmation of our study results.

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Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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