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Indications and Complications of Prenatal Invasive Diagnostic Tests: A Retrospective Descriptive Study

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ABSTRACT

Background & Objective: Prenatal invasive procedures are used for diagnostic and therapeutic purposes. The present study aimed to assess the indications and early complications of invasive diagnostic tests.

Materials & Methods: This retrospective descriptive study was conducted on 708 pregnant women who were referred for prenatal invasive tests in Tehran, Iran from May 2018 to April 2022. All medical records of the participants were reviewed and entered into the study. According to the implemented procedures, medical records were categorized into two chorionic villus sampling (CVS) and amniocentesis groups. The primary outcome was determining the frequent indications of invasive diagnostic tests and post-procedure complications.

Results: Six hundred and sixty-eight medical records were included. Amniocentesis procedure was performed for 624 (93.7%) and 44 cases (6.3%) underwent CVS. The most frequent indication for invasive prenatal procedures was a history of abnormal findings related to the first trimester biomarkers followed by the abnormal findings of the second trimester biochemical markers, a history of high nuchal translucency>99th percentile, and abnormal biomarkers of sequential test. Comparing post-procedure complications, the results showed no significant difference between the CVS and amniocentesis groups (P=0.845). In the amniocentesis group, 2 cases had shown spontaneous abortion and 3 leakage of amniotic fluid, as well as two cases in the CVS group, had reported vaginal bleeding.

Conclusion: Our results delineated that positive fetal aneuploidy screening tests together with increased nuchal translucency were the main indications of the invasive prenatal tests. Amniotic fluid leakage, vaginal bleeding, and spontaneous abortion should be considered as procedure-related complications.

Keywords: Chorionic Villi Sampling, Amniocentesis, Diagnostic Test, Pregnancy

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Introduction

Currently, prenatal invasive procedures are used for diagnostic and therapeutic purposes. Amniocentesis is one of these techniques that was introduced in 1956 **(1, 2)**. It is usually performed after 15 weeks of gestation to attain amniotic fluid and fetal cells for biochemical, and chromosomal study **(2-4)**. Since the 1970s, chorionic villus sampling (CVS) has also been implemented to collect small samples of the placenta for earlier cytogenetic evaluation **(5)**. CVS is generally done after 10 weeks of gestation **(6)**.

Although these invasive techniques are performed under real-time ultrasound guidance to provide adequate visualization and reduce the risks of fetal injuries **(7)**, post-procedure complications may threaten maternal and fetal lives. Amniotic fluid leakage, vaginal spotting, miscarriage, fetal loss, uterine contraction, chorioamnionitis, admission in hospital, preterm birth, and perinatal mortality are some of these complications **(4, 6)**. There are few case-report studies showing post-procedure septic shock **(8, 9)**. Fortunately, evidence showed that these complications are not frequent. Among 250566 pregnant women who underwent amniocentesis, the incidence rates of rupture of membrane, fetal death, infection, and spontaneous abortion within two weeks were 0.24%, 0.11%, 0.05%, and 0.05%, respectively. The rates of intrauterine fetal death and spontaneous abortion related to post-CVS procedure among 1409 cases were also reported 1.68% and 0.77%,

respectively **(10)**. Furthermore, the incidence of chorioamnionitis and infection after invasive tests are below 0.5% **(9)**.

Concerning the indications of prenatal invasive tests, it was reported that advanced maternal age and positive screening tests for chromosomal aneuploidies in the first and second trimesters were the main indications. Moreover, autosomal trisomy accounted for about half of chromosomal abnormalities **(11-14)**. On the other hand, recent studies have shown a decrease in the indications related to advanced maternal age and an increase in the indications due to the risks of genetic disorders and ultrasound abnormal findings **(15-17)**.

The American College of Obstetricians and Gynecologists (ACOG) emphasized the availability of invasive prenatal tests for all pregnant women, regardless of their age or risks **(18)**. Accordingly, a significant number of pregnant women at risk of fetal cytogenetic abnormalities are referred to our tertiary referral center for invasive prenatal tests.

Hence, the present study aimed to assess the indications and early complications of invasive diagnostic tests in our perinatology center within four years. The results of this study will provide informative and comparative data.

Methods

This retrospective descriptive study was conducted on pregnant women who had been referred for prenatal invasive tests (CVS or Amniocentesis) at Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran from May 1, 2018 to April 30, 2022. All Medical records of 708 participants were reviewed and entered into the study. The included subjects had an ultrasound examination before performing the procedures to assess fetal viability, gestational age, and the need for invasive diagnostic procedures. The procedures were performed for them by perinatology fellows under the supervision of faculty members with transabdominal ultrasound guidance. No trans-cervical CVS procedure or cordocentesis was carried out for the included population. Anti-D immunoglobulin was administered for Rh-negative mothers at risk of alloimmunization.

Inclusion criteria were CVS procedure at 10-13 weeks and amniocentesis at 15–19 weeks of gestation and availability of data related to follow-up visits for at least two weeks after the procedures. Medical records without ultrasound examination, no follow-up information, or missing data were excluded (n=40) from the study.

According to the implemented procedures, all collected medical records were categorized into two CVS and amniocentesis groups. Participants' demographic, obstetrical, and clinical characteristics, including maternal age, the history of any underlying disease, gestational age (based on the first day of the last menstrual period), the numbers of gravidity, parity, abortion, the type of blood group, the number of gestations, and the history of aneuploidy in previous pregnancies were determined and extracted from the medical records. Furthermore, other data related to the current pregnancy, such as the results of prenatal screening tests, size of Nuchal Thickness (NT), the result of a cell-free DNA test, the ultrasonography findings, gestational age at the time of the procedure, the indications of the invasive procedures, and the consequent complications (rupture of membrane or leakage of amniotic fluid, spotting, spontaneous abortion, intrauterine fetal death, and chorioamnionitis) within two weeks after the implemented procedure were collected and recorded.

Primary/secondary outcome

The primary outcome was determining the frequent indications of invasive diagnostic tests in our center. To determine early prenatal outcomes after invasive diagnostic tests, all complications were compared between the groups as the secondary outcome.

Ethical consideration

The present study was approved according to the Helsinki Declaration by the Ethics Board of the Tehran University of Medical Sciences (ID: IR.TUMS.MEDICINE.REC.1401.189).

Written consent was received from included participants and all data was considered confidential.

Sample size

All candidates for invasive prenatal tests during four years were determined as the sample size.

Statistical Analysis

The collected and recorded data were analyzed using the Statistical Package for the Social Sciences, version 23.0, SPSS Inc., Chicago, Illinois, USA (SPSS). Mean ± standard deviation and number (%) were used to show the quantitative and qualitative variables, respectively. Analyses of the relationships between the quantitative variables were performed by the Independent student t-test. The significant level was considered p-values less than 0.05.

Results

Six hundred and sixty-eight medical records of singleton and twin pregnancies were included. The mean age of included subjects was 33.62±6.03 years (14–49 years). Concerning maternal underlying disease, gestational diabetes mellitus was reported in a mother (0.3%). Eighty-two mothers (11.8%) had a past history of abortion. About 98% had spontaneous conception and 1.9 % had ART (assisted reproductive techniques) pregnancy. Of those pregnant women were referred for invasive prenatal tests, 216 cases had abnormal findings related to the first screening test. Abnormal findings had been also reported in 153 and

84 participants with the second and sequential screening tests, respectively (Tables 1). Amniocentesis procedure was performed for 624 (93.7%) and 44 cases (6.3%) underwent CVS. No early amniocentesis (before 15 weeks) had been done, and the mean gestational age at performing this procedure was 16±3 weeks. The mean gestational age at the CVS procedure was also 12±4 weeks.

As data are shown in Table 2, the most frequent indication for invasive prenatal procedures was a history of abnormal findings related to the first trimester biomarkers followed by the abnormal findings of the second trimester biochemical markers, increased NT>99th percentile, and abnormal biomarkers of sequential test. According to the results of screening tests, trisomy 21 was the most common autosomal aneuploidy in the first and second-trimesters. Fetal anomaly reported by ultrasound examination was also the reason for 11.5% of performed invasive tests. Of them, brain (n=11) and heart (n=10) abnormalities were the most frequent indications for invasive tests. Of 27 cases with high-risk cell-free DNA results, 81.48% were at risk of trisomy 21.

Comparing post-procedure complications, the results showed no significant difference between the CVS and amniocentesis groups (P=0.845). In the amniocentesis group, two cases showed spontaneous abortion and three transient leakages of amniotic fluid, as well as two cases in the CVS group, reported vaginal bleeding. Abortion and leakage of amniotic fluid were not observed in the CVS group (Table 3). The leakage of amniotic fluid after amniocentesis in our cases discontinued during two weeks.

Table 1. The mean, Standard deviation, median, minimum, and maximum concentrations of biomarkers and final results of the first, second, and sequential screening biomarkers test

Values	*First-trimester biomarkers*		*Second-trimester biomarkers*				*Sequential biomarkers*					
	PAPP-A (MOM)	FβHCG	βHCG	AFP	Inhibin - A	UE_3	PAPP-A (MOM)	FβHCG	βHCG	AFP	Inhibin -A	UE3
Number	357	357	115	115	111	115	86	86	86	86	86	86
Mean	0.880	2.283	1.989	1.054	1.871	0.759	0.997	2.283	2.180	1.301	2.142	0.689
Median	0.700	1.915	1.580	0.83	1.570	0.695	0.780	1.460	1.825	0.940	1.810	0.620
SD	0.648	1.644	1.520	0.729	1.205	0.309	0.711	3.180	1.547	1.377	1.194	0.310
Min	0.05	0.06	0.14	0.37	0.25	0.25	0.16	0.18	0.15	0.30	0.50	0.14
Max	4.34	9.64	9.14	5.39	7.91	2.08	5.32	24.95	11.53	8.60	6.96	1.97
Low-risk	44 (12.2)			8 (5.4)					2 (2.3)			
High-risk	216 (87.8)			153 (94.6)					84 (97.7)			

Min: minimum; Max: maximum; SD: standard deviation; AFP: alpha fetoprotein; UE3: unconjugated estriol; B HCG: beta human chorionic gonadotropin

Table 2. Indications of amniocentesis or chorionic villus sampling procedures

Indications	Number	Percent
Positive First-trimester biomarkers	216	32.3
Positive Second-trimester biomarkers	153	22.9
Positive Sequential biomarkers	84	12.5
Increased NT (>99thpercentile)	97	14.5
Fetal anomaly	77	11.5

Indications	Number	Percent
High-risk cell-free DNA test	27	4.04
History of aneuploidy and metabolic disease in previous pregnancy	6	0.89
Viral infections	3	0.44
Genetic disorder of parents	4	0.59
Parent's request	1	0.14

NT: nuchal translucency

Table 3. Complications during two weeks after procedures

Complication	Amniocentesis group n=624	CVS group n=44	P value
Abortion	2 (0.32 %)	0	0.845
Leakage of amniotic fluid	3 (0.48 %)	0	
Vaginal bleeding	0	2 (4.54%)	

Independent student t-test

Discussion

Concerning the prevalence of fetal chromosomal anomalies, serum biomarkers or cell-free DNA is evaluated during the first trimester of pregnancy. Based on these primary aneuploidy screening tests, cytogenetic analyses may also be needed for the determination of at-risk mothers. Furthermore, diagnostic genetic testing should be available to all pregnant women who desire in regardless of the risk factors. The present study was conducted to demonstrate the frequency of performing these diagnostic procedures, their indications, and short-term consequences of 668 pregnant women who were referred to our center during four years. The results showed that positive tests related to the first and second trimester's screenings accounted for the high percentages of indications. Moreover, amniotic fluid leakage, vaginal bleeding, and spontaneous abortion should be considered procedure-related complications.

According to the results, the amniocentesis procedure was performed for 93.7% of subjects and 6.3% of the participants underwent CVS. Although prenatal invasive methods are implemented based on indications, gestational age, and the urgency of obtaining the results **(4)**, it was reported that amniocentesis is the most frequent technique in detecting fetal genetic disorders **(19, 20)**.

Based on the results, the mean age of our subjects was 33.62±6.03 years. This finding shows that advanced maternal age was not a main indication for invasive procedures. This value was very close to the reported mean ages by the other studies from Iran. Younesi and co-workers showed that the mean age of 15401 participants who underwent amniocentesis was 33.3±5.8 years **(21)**. Tara and colleagues also demonstrated that the mean age of 1000 pregnant women performing amniocentesis was 33.4±6.0 years **(19)**.

According to the results, the mean gestational age at performing amniocentesis was 16±3 weeks. It is supposed that after 15 weeks is the best time for amniocentesis due to complete fusion of amnion and chorion membranes. Nicolaides and co-authors showedthat the spontaneous loss rate after early amniocentesis (before 15 weeks) was significantly higher than after CVS (5.8% vs. 1.8%) **(22)**.

In the present study, the mean gestational age at the transabdominal CVS procedure was 12±4 weeks. The benefit of CVS is earlier detection of fetal genetic abnormality compared to amniocentesis. CVS procedure could be performed transabdominal or trans cervical at 10–13 weeks of gestation depending on the gestational age, the position of the uterus and bladder, and placental localization; however, very early CVS may increase the risks of congenital defects **(23)**.

Concerning the indications of prenatal invasive tests, screen positive tests related to the first and second trimesters screening accounted for the high percentages of indications of invasive procedures. Furthermore, the risk of trisomy 21 was the most common abnormal result of aneuploidy analyses. The results also demonstrated that NT>99th percentile was a notable ultrasound finding among our participants who underwent prenatal invasive tests. Confirming our results, it was reported that an increased NT (measured between 11 and 13^{+6} weeks of gestation) together with abnormal findings related to two first screening tests can show an individualized risk of autosomal aneuploidies that needs genetic study by invasive

prenatal test **(24)**. In accordance with our findings, Lichtenbelt and co-workersshowed that ultrasound and screening tests findings were the majority of the indications for performing prenatal invasive tests among pregnant women <36 years during 2000-2009. The authors also demonstrated a high rate (71%) of detected autosomal trisomies with notable dominance for trisomy 21 among 10706 pregnant women who underwent invasive procedures **(25)**.

Regarding the post-procedure complications, our results indicated that 0.4% and 0.3% of all cases who underwent amniocentesis had leakage of amniotic fluid and spontaneous abortion, respectively. Accordingly, it seems that amniotic fluid leakage and spontaneous abortion should be noticed as the main complications after amniocentesis. Our finding related to the rate of post-amniocentesis miscarriage is similar to the reported rate (0.3%, 95% CI, 0.11-0.49) by a systematic review in 2019 (26).Comparing the rates of amniotic fluid leakage during two weeks post-procedure, Sanei Sistani and Tardast **(27)** reported a higher rate of amniotic fluid leakage by 2% among 100 Iranian pregnant women **(27)**. This difference may be related to the earlier procedure compared to our study (15^{+4} vs. 16 weeks), implemented technique, or operator experience, and difficult procedures.

It should be also noted that post-procedure amniotic fluid leakage in our cases was stopped before two weeks. The other studies also pointed out that post-procedure leakage usually relieves after three weeks (range 1-7 weeks). Reaccumulation of amniotic fluid and changes in the decidua and myometrium block further leakage and prevent the adverse prenatal outcomes **(28)**.

Although spontaneous abortion and leakage of amniotic fluid were not observed in the CVS group, 4% of the cases had transient vaginal bleeding. As the CVS method in our study was performed after 10 weeks, this may explain the cause of no reported fetal loss or rupture of membrane. In accordance with our findings, an investigation by Sanei and co-authorsdemonstrated Sanei Sistani and Tardast **(27)** the same incidence rate of vaginal bleeding during 14 days of the follow-up period among 50 cases with transabdominal CVS (27). Bakker, Birnie **(20)** demonstrated that besides gestational age at the performing procedure, other factors like the type of CVS (transabdominal or transvaginal), the number of CVS attempts, the implemented devices for sampling (forceps or cannula), and operator experience can significantly affect the incidence of post-CVS complications **(20)**.

Comparing the post-procedures complications, our results showed no significant difference between the CVS and amniocentesis groups. Accordingly, it is supposed that both diagnostic invasive procedures can be implemented in high-risk pregnancies. Another study from Iran also confirmed this result and showed no statistically significant differences in the incidence rates of vaginal bleeding (1% & 4%; p=0.465) and leakage of amniotic fluid (2% & 2%; p=0.632) among the CVS (n=50) and amniocentesis (n=100) groups **(27)**. Mujezinovic and colleaguesalso showed that transabdominal CVS and amniocentesis had similar risk of fetal loss rate **(29)**.

The findings of the present study showed that the abnormal first and second trimesters biochemical screening results and ultrasound findings related to NT>99^{th} percentile were the main indications for prenatal invasive procedures. Although transient vaginal bleeding, amniotic fluid leakage, and spontaneous abortion were the main complications after CVS and amniocentesis, no significant differences were observed between the CVS and amniocentesis groups regarding these complications. It should be also noted that recent study had several limitations including small sample size in the CVS group and different operators with different experiences.

Conclusion

Our results delineated that positive fetal aneuploidy screening tests together with increased nuchal translucency were the main indications of the invasive prenatal tests. Considering no significant differences in the rate of post-procedures complications between the amniocentesis and CVS groups, both procedures can be implemented in high-risk pregnancies. Amniotic fluid leakage, vaginal bleeding, and spontaneous abortion should be considered procedure-related complications. Further studies with larger sample sizes are needed to confirm our results.

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Authors' Contributions

Dr. Ghazi, Dr. Ashraf Jamal, Dr. Marsoosi, and Dr. Eslamian carried out the design, coordinated the study, and participated in most of the experiments. Dr. Nurzadeh, Dr. Naemi, Dr. Shariat, and Dr. Boustani provided assistance for all experiments, analysis of data, and participated in manuscript preparation. All authors have read and approved the content of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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