International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Kazal RK et al. Int J Reprod Contracept Obstet Gynecol. 2021 Jan;10(1):50-54
www.ijrcog.org

pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20205753

Original Research Article

Second trimester amniocentesis for prenatal diagnosis of genetic disorders in Bangladesh

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Received: 05 October 2020
Revised: 16 November
Accepted: 01 December 2020

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ABSTRACT

Background: Amniocentesis is characteristically carried out under ultra-sonographic control, between 15 and 17 weeks of pregnancy. The term prenatal diagnosis firmly comprises all diagnostic modalities aimed at gaining information about the embryo. Its history includes the development of cytogenetic, molecular genetics and molecular cytogenetic methods. Prenatal diagnosis is now possible for a considerable number of genetic diseases and/or birth defects using a variety of techniques. Objective was to evaluate the safety, feasibility and outcome of second trimester amniocentesis for prenatal diagnosis of genetic disorders.
Methods: This was a descriptive study, conducted at fetal medicine center, Family Care Foundation, Dhaka, Bangladesh from June 2014 to December 2019. A total of 350 pregnant women had undergone 15-20 week's transabdominal amniocentesis under real-time ultrasound guidance. A 23 gm/ 88 mm spinal needle was used. The needle was passed though the maternal abdomen into the amniotic cavity in its longitudinal direction. Once the needle was adequately placed, the amniotic fluid is aspirated with a suction force through a 20 cc syringe. All amniocentesis was performed with "two operators" technique.
Results: A total of 350 Amniocentesis were done. Beta thalassemia was most common (79.7%). Followed by aneuploidy (10.2%), hemophilia (6.2%), SMA (2.0%), DMD (1.7%), hematoma (7%), intra amniotic bleeding (2%) and per vaginal bleeding (2.8%). 3.7% aspiration was difficult due to fibroid and retroverted uterus. The overall aspiration success rate was 100%.
Conclusions: Second trimester transabdominal amniocentesis in an outdoor setting with the help of real-time sonography is a safe procedure with no significant risk to the mother and the fetus.

Keywords: Amniocentesis, Amniotic, Diagnosis, Needle, Prenatal

INTRODUCTION

Amniocentesis is an invasive procedure of prenatal diagnosis which means the extraction of amniotic fluid from the womb with the help of a needle. Amniocentesis was first performed for genetic studies in the 1950s. Serr and colleagues were the first to report the use of amniocentesis for antenatal sex determination.[1] Prenatal diagnosis had its beginning in 1966, when Steele and Breg cultured amniotic cells and analysed their karyotypes. Subsequently, amniocentesis has been performed for the diagnosis of a variety of disorders including chromosome abnormality.[2] Still in modern practice, second trimester amniocentesis remains the most popular technique for fetal genetic assessment. This method has been accepted as safe as well as accurate due

to real time monitoring of the needle by ultrasound.[3] Following the introduction of cytogenetical diagnosis- the prenatal diagnosis from amniotic fluid has become a reality and is a permanent challenge for obstetricians. This procedure should be made available to pregnant women who may require prenatal diagnosis of genetic disease. Perinatal morbidity and mortality in Bangladesh from genetic disorders are not uncommon. Most of such disorders are either not treatable or the cost of treatment, if available, is out of the reach of the common population. Inherited genetic diseases like thalassemia are a great burden in our Bangladesh.[4,5] On the other hand, WHO reports that about 5500 children are born with Down's syndrome each year in our Bangladesh.[6] Prenatal diagnosis of affected fetus and discontinuation of early pregnancies is an important option for the elimination of genetic disorders like thalassemia and Down's syndrome. Study on amniocentesis is first of its kind to report in Bangladesh. The objective of this study was to determine the safety, feasibility and outcome of second trimester transabdominal amniocentesis for the prenatal diagnosis of common genetic/chromosomal disorders.

METHODS

From June 2014 to December 2019, a total of 350 couples requested prenatal diagnosis for various genetic disorders. All are with singleton pregnancies. Couples were counseled about the indications, other options, complications and errors in diagnosis before starting the procedure. In case of affected fetus, the termination of pregnancy and its religious implications are also discussed. A written consent was taken from all couples. We determined to perform amniocentesis in the early second trimester after 15 weeks. The pregnant mothers came with prior appointment according to their gestational age. At first, an ultrasound scan was performed to check the fetal viability, age of gestation, number of fetus, placental position and any other incidental findings that may have any impact on the procedure. All the procedures were performed via transabdominal route using a 3.5 MHz convex probe. Placental position was checked and a suitable site for passing the needle though the anterior abdominal wall was selected. 10% povidone iodine solution was applied on the abdominal skin for as disinfectant. Under real time ultrasound guidance, a 23 G, 88 mm Spinal needle (B Braun, Germany) (Figure 1), was inserted though the abdominal wall, seen traversing the uterine wall and placed into the amniotic cavity (Figure 2). With the needle in place, the amniotic fluid was aspirated with a suction force through a 20 cc syringe. The needle tip was kept visible all times during the procedure as an important step. In the cases with the first negative puncture we tried a second one during the same intervention, but if this second procedure was also negative (no amniotic fluid has been removed), we repeated the procedure after 1-2 weeks. The initial 1-2 ml of amniotic fluid was discarded to prevent contamination with maternal cell and we aspirated around 15 ml of amniotic fluid (1 ml amniotic fluid/week). The sample was send immediately to the genetic laboratory at the ambient temperature. We adopted the "two operator's technique" during the whole procedure, (Figure 3), one for manipulating the ultrasound probe and creating the image and other for needle maneuvers. The average time from insertion of the needle to aspiration of amniotic fluid was about 10 minutes. Anti D immunoglobulin was administered to all Rh negative mothers in the first 24 hours after amniocentesis. The puncture mark was sealed with a sterile waterproof bandage. A post-aspiration USG scan was done to see the fetal wellbeing, any haematoma formation, or placental separation. After 30 minutes to one hour after the procedure, the patients were allowed to go home. Advices are also given to take bed rest for at least 24 hours and avoid travelling for 3 days. Prophylactic antibiotics of cephalosporin group and paracetamol tablets were advised for pain relief. A follow-up was done after one week to check any complication. Genetic analyses of samples were done in DNA Solution, Dhaka, Genetic Lab of Dhaka Shishu Hospital, Medical genetics Lab of Sir Ganga Ram Hospital, New Delhi, India. The report of prenatal diagnosis, ethical issues related to possible termination of pregnancy to follow if any, was discussed with the couples. Descriptive statistics were applied to the data using software SPSS 22.0 version.

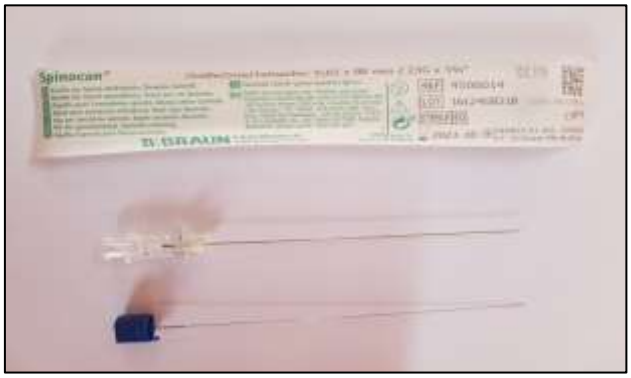

Figure 1: 23 G, 88 mm spinal needle (B Braun, Germany).

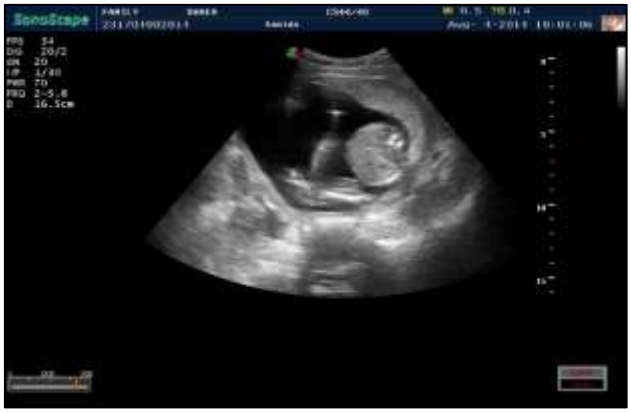

Figure 2: Clear view of needle tip inside the amniotic cavity.

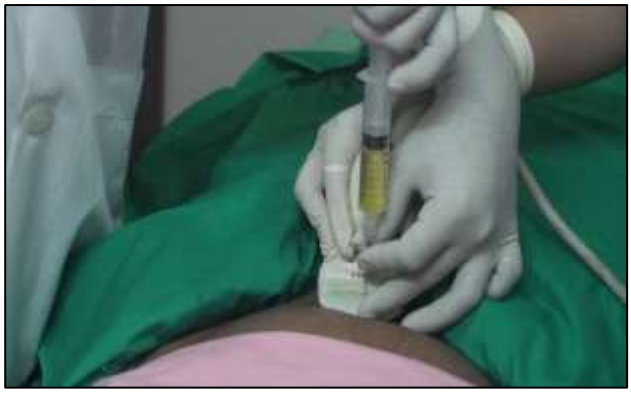

Figure 3: "Two operators' technique", one for manipulating the ultrasound probe to create the image and other for needle maneuvers.

RESULTS

A total of 350 second trimester amniocentesis procedures were done for various indications during the study period. Maximum age group of mother, 134 out of 350 (38.2%) was 25-30 years (range 20-40 years). Most procedures (89.1%) were done between 15 and 17 weeks (range 15-20 weeks) (Table 1). Indications and results of the procedure are shown in Table 2. The most common indications of prenatal diagnosis were for beta-thalassaemia/ e-beta thalassemia (79.7%) which included 279 couple. Other indications were for diagnosis of aneuploidy (10.2%), hemophilia (6.2%), spinal muscular atrophy (SMA) (2.0%), Duchenne muscular dystrophy (DMD) (1.7%). All the couples referred for prenatal diagnosis by amniocentesis had a history of one or more affected children with their respective indication.

Table 1: Maternal characteristics.

Parameters	Frequency (n=350)	Percentage
Age (years)		
20-24	99	28.2
25-30	135	38.5
31-35	81	23.1
36-40	35	10.0
Gestation age (weeks)		
15-17	312	89.1
18-20	38	10.8

Table 2: Indications and results of the procedure (N=350).

Indications types and frequency	Affected N (%)	Carrier N (%)	Normal N (%)	Total N (%)
Both parents are carrier of haemoglobin disorder (beta trait/Hd E trait)	59 (21.1%)	137 (49.1%)	83 (29.7%)	279 (79.7%)
Aneuploidy check	2 (5.5%)	--	34 (94.4%)	36 (10.2%)
Hemophilia	4 (18.1%)	8 (36.3%)	10 (45.4%)	22 (6.2%)
Duchenne muscular dystrophy (DMD)	2 (28.5%)	3 (42.8%)	2 (28.5%)	6 (1.7%)
Spinal muscular atrophy (SMA)	1 (16.6%)	3 (50.0%)	2 (33.3%)	7 (2.0%)

Results of the 279 samples for prenatal diagnosis of beta thalassemia, affected babies were 59 (21.1%), carrier 137 (49.1%), and normal (no mutation in beta chain) 8 (29.7%). In 36 case of chromosome check, only 2 (5.5%) found abnormal. One of which was trisomy 21 and the other was a case of Klinefelter syndrome (47, XXY). The other 24 (94.4%) were of normal karyotype. The result of prenatal diagnosis in 22 case of hemophilia were affected fetuses 4 (18.1%), carrier 8 (36.3%), normal 10 (45.4%). Out of 6 prenatal diagnosis of Duchenne muscular dystrophy (DMD), only 1(16.6%) fetus was affected, carrier, 3 (50.0%), and normal 2 (33.3%). In case of other autosomal recessive genetic disorder, we 7 couple were referred to us for prenatal diagnosis of spinal muscular atrophy (SMA). Out of 7, 2 (28.5%), fetuses were normal, 3 (42.8%), were carriers and 2 (28.5%) fetuses were affected. Aspirations of amniotic fluid were easy and in most cases (96.2%), adequate sample collection was possible in first attempt, however, in 3.7% cases, the aspiration was difficult due to a variety of factors like fibroids in anterior uterine wall and retroverted uterus. These mothers were called for a repeat procedure one week later which were successful. The overall success rate of aspiration was 100% (Table 3). Minor complications like placental hematoma and minor intra-amniotic bleeding, slight per vaginal occurred in 7.1%, 2% and 2.8% cases respectively which were subsided by conservative management (Table 4). Any miscarriage related to the procedure, within 3 weeks not occurred in any cases.

Table 3: Outcome of procedure (N=350).

Parameter	Frequency	Percentage
Successful at first attempt	337	96.2
Repeat procedure after 1 week	13	3.7

Table 4: Procedure related complications (N=350).

Parameters	Frequency	Percentage
Placental hematoma	25	7.1
Intra amniotic bleeding	7	2
Per vaginal bleeding	10	2.8
Miscarriage within 3 weeks	0	0.0

DISCUSSION

Amniocentesis was a "blind" procedure up to mid 1970s.[7] Doctors Jens Bang and Allen Northeved from Denmark were the first to report amniocentesis done with the guide of an ultrasound in 972.[8] Prenatal diagnosis through early fetal sampling has played a pivotal role in the prevention of genetic disorders.[9] Ultrasound guidance adds to the safety for the fetus as well as the mother. Moreover, rapid analytic techniques have significantly reduced the waiting time between sampling and diagnosis which is very important reason that if it is to be followed by termination of pregnancy then it should be done within a reasonable timeframe defined by consensus. Nevertheless, an elaborate learning process to master the technique of amniocentesis remains indispensable.[10] In this study majority of the procedure were done between 15-17 weeks. We used 23 G, 88 mm spinal needle (B Braun, Germany) as it is much cheaper and easily available. Majority of invasive prenatal diagnostic procedures in the west are performed for individuals deemed to be at high risk for Down's syndrome. Sebija et al performed 299 amniocentesis procedures at 16-20 weeks gestation whose primary indication was chromosomal anomalies and single gene defects in 84.9% and 5% of cases respectively.[11] In our study majority of amniocentesis procedures were done to pick up thalassemia major fetus to allow timely termination in early 2nd trimester at a time when complication rate along with maternal tension is decreased due to early voluntary termination of pregnancy.[12] In our study, in contrast to western studies, only 9.7% procedures were done to detect chromosomal anomalies.[13] All of these mothers had previous babies with Down's syndrome. Identifying high risk pregnant mother by first trimester screening and referral for prenatal diagnostic procedure for aneuploidy by amniocentesis is yet to be well practiced in our perspective. ACOG suggests that it should be advised to all pregnant women for aneuploidy screening before 20 weeks, and should be offered diagnostic testing regardless of their age or other risk factors.[14] Like the study in Pakistan by Suhaib Ahmed,[15] other indications in our study were Hemophilia, DMD, and SMA. So, not only Hemoglobinopathies, other inherited genetic disorders are also prevalent in our country due to high prevalence of consanguineous marriage. Aspirations were easy and in most cases adequate sample collection was possible in first attempt (96.2%). In rest of the cases (3.7%) successful sample collection was done one week later. Due to some factors like fibroids in anterior uterine wall, retroverted uterus, aspiration was sometimes difficult. High body mass index was not a limiting factor in amniocentesis procedure. The overall aspiration success rate was 100%, which is similar to the study of 144 amniocenteses by Ahmed, and higher success rate than the study of Tamimi, who reported the success rate of 96.8%.[15,16] Bleeding and spotting are not very common, although Abeera et al have reported haematoma formation in 1.5% cases and vaginal bleeding in 0.5% cases.[17] We experienced the same minor complications like placental hematoma, per vaginal bleeding, minor intra-amniotic bleeding occurred in 7.1%, 2.8% and 2% cases respectively which were subsided by conservative management. Most serious complication after amniocentesis is pregnancy loss. According to Centers for disease control and prevention (CDC), the risk of miscarriage has been attributed to 0.25%-0.50% of amniocentesis procedures.[18] Data from systematic reviews and randomized controlled trials are consistent with a procedure-related risk of miscarriage at a rate of 0.5-1.0% for amniocentesis as well as for chorionic villus sampling (CVS). Amniocentesis performed prior to 15 weeks had a significantly higher miscarriage rate and may have risk of talipes equinovarus.[19,20] But this was not an issue in our study as all the procedures were carried out between 15-20 weeks. Washington University School of Medicine published data of 58,436 women who undergone such procedures from 1990-2006. There the fetal loss rate occurred in 0.97% of the amniocentesis group and 0.84% of the group who had not undergone any procedure. The institutional fetal loss rate attributable to amniocentesis is 0.13% or 1 in 769 which was not significantly different from that observed in patients who had undergone no procedure.[21]

In our study, there were no procedure related miscarriages occurred in any case. We followed the cases physically or over telephone. Most women had mild to moderate pain following the procedure which settled with paracetamol tablets. The results of our study in terms of miscarriage and other maternal morbidity conform to the internationally accepted data. Although in a single-center study performance may be remarkably good due to very skilled operators, we think our study will help in counselling the prospective parents considering the use of amniocentesis and about the benefits, risks, limitations of these procedures.

CONCLUSION

Real-time ultrasound guided second trimester transabdominal amniocentesis is a useful, safe and reliable outdoor procedure for fetal sampling and prenatal diagnosis in early pregnancy and should be considered as a procedure of choice. In experienced hands, the miscarriage rate is very low, thus, it can be safely offered for prenatal diagnosis. It can play an important role in the prevention of genetic disorders and reduce the burden of the diseases that are otherwise incurable. We realized the high response and acceptance of the Bangladeshi couples to prenatal diagnosis and termination of pregnancy.

ACKNOWLEDGMENTS

The author wishes to acknowledge the technical blessing and support by Dr. I. C. Verma, Dr. Ratna Dua Puri of Genetic Medicine Department and Dr. Ashok Baijal, Dr. Nandita Dimri Gupta of Fetal Medicine Department, Sir Ganga Ram Hospital, New Delhi, India.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Kazal RK, Chowdhury SA, Pervin T, Pervin HH, Kaniz PW. Second trimester amniocentesis for prenatal diagnosis of genetic disorders in Bangladesh. Int J Reprod Contracept Obstet Gynecol 2021;10:50-4.