RESEARCH ARTICLE

Fetal Blood Sampling: Indications, Outcome and Complications

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Introduction

The fetus has been given the status of an individual "patient" in whom various procedures for the diagnosis and even therapy of certain congenital and genetic diseases can be performed by fetal blood sampling.[1] Fetal blood sampling is a science of identifying various causes for fetal abnormalities like chromosomal, genetic, hematological, infections, immunological, and physiological assessment of fetus and when other prenatal diagnostic procedures fail. Fetal blood sampling refers to three techniques used to gain access to fetal blood,[2] namely:

- Cordocentesis (also known as funiculocentesis or percutaneous umbilical blood sampling).
- Intrahepatic portion of umbilical vein sampling.
- Cardiocentesis.

Materials and Methods

This is a prospective study done on referred patients from all over South India to Bhagwan Mahaveer Jain Hospital and the Bangalore Prenatal Diagnosis and Therapy Associates, Bengaluru. It was carried out on pregnant women with at least one indication for fetal blood sampling. A total number of patients in the study period of 12 months were enrolled irrespective of age, parity, period of gestation, and type of indication.

Inclusion Criteria

- Previous offspring with chromosomal anomalies or other birth defects.
- Maternal age 35 years or more at first pregnancy.
- Recurrent pregnancy loss.
- Suggestive fetal ultrasonographic findings.
- Positive maternal screening test findings.
- Pregnant woman/spouse has a family history of mental retardation/physical deformities/genetic disease/inborn errors of metabolism.
- Mother exposed to drugs, medications, or infections known to be associated with congenital malformations in the fetus.
- Any indication for fetal therapy.

Exclusion Criteria

- History of bleeding diathesis or hematological disorders.
- Intrauterine death of the fetus.

Procedure

All eligible patients have signed the informed consent form. Patient had to sign Form G and Form F as per the PC and PNDT Act 2003. History and investigations were done as per the protocol. Under aseptic precautions, the procedure is performed under ultrasound guidance with continuous needle tip visualization. About 2 mL of fetal blood is obtained and sent for chromosomal, DNA, or biochemical analysis. If indicated, fetal therapy is performed. Following the procedure, further, follow-up was done until the outcome of pregnancy. Post-procedure antibiotics were given, and the patient was referred back to her primary obstetrician. The patients were followed up to the end of pregnancy. The outcome of the pregnancy and its relation to indication, complications, and results were analyzed.

Technique of Performing Cordocentesis

It is based on the direct ultrasonographic guidance of a needle. It is performed after 18 weeks of gestation. The patient is prepared as if for an operative procedure. Placental location and insertion of the cord are defined. A local anesthetic is administered in the area where the needle is to be inserted into the maternal abdominal wall. A 10–16 cm spinal needle is used based on the measured distance from the skin surface to the targeted segment of the umbilical cord. 22 gauge needle is most widely used. Ideally, the umbilical cord is punctured 1–2 cm from its placental insertion. Either the umbilical vein or artery can be sampled with apparently equal success and safety, although there is some concern about vascular spasm and fetal bradycardia after arterial puncture. If fetal therapy (e.g., transfusion) is contemplated, entering the fetal umbilical artery is preferred. 0.5 cc of fetal blood is aspirated into a small heparinized syringe and discarded to avoid maternal contamination. The diagnostic sample (0.5–3 cc) is then aspirated into a new heparinized syringe, immediately labeled, and transported for analysis.

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Advantages

- Cordocentesis is the method of choice for life-saving diagnostic and therapeutic measures in erythroblastosis fetalis.
- Another potential indication is an assessment of the fetal platelet count in cases of alloimmune thrombocytopenia and in idiopathic thrombocytopenia purpura.
- It can be used for fetal therapy including intravascular transfusions and introduction of medications for fetal treatment.[3,4]
- Fetal hypoxia can be detected by measuring the fetal blood gases, pH, and lactate with this method.
- Karyotyping can be done when fetal congenital malformations or intrauterine growth retardation are identified by ultrasound.
- Prenatal diagnosis of viral infections tox—oplasmosis, CMV, and rubella.
- Prenatal diagnosis of inborn errors of metabolism.
- Prenatal diagnosis of hemoglobinopathies.
- Cordocentesis may be used to sample another cell line in cases with possible fetal mosaicism.
- Karyotyping can be completed in 7 days from fetal blood, this time can be critical if the time limit for therapeutic abortion is near and also helps in early diagnosis of various chromosomal translocations by providing faster detailed karyotype report. Fluorescence *in situ* hybridization (FISH) can be performed on fetal blood, and reports will be available in 3 days.
- Many genetic disorders are not expressed in cells obtained by amniocentesis or chorionic villus sampling and require fetal blood for analysis.
- Fetal blood analysis is required following amniotic fluid culture failure/failed chorionic villus sampling.
- As the risk of obtaining fetal blood has decreased, the indication for this procedure has grown, making it a much more widely used investigation.

Risks and Complications

Pregnancy loss, fetal bradycardia, bleeding, and others like preterm labor, preterm premature rupture of membranes, and uterine infections including chorioamnionitis.

Technique of Performing Cardiocentesis

The technique of cardiocentesis resembles that of umbilical cord puncture. Fetal cardiocentesis may be a reasonable option to obtain fetal intravascular access and facilitate therapeutic interventions when cordocentesis fails or is not feasible. However, the expected benefit must clearly outweigh the risk of fetal loss (5%) and if two-needle insertions are required for a successful cardiocentesis or if cardiocentesis follows failed funipuncture, the fetal loss rates may be as high as 14.3% and 15.4%, respectively.[5]

A four-chamber view of the fetal heart should be obtained and the maternal abdomen prepared. A 22-gauge, 9-cm spinal needle is guided to the right ventricle of the fetal heart under continuous ultrasound visualization. The right ventricle is chosen because it lies closer to the anterior chest wall. Once the tip of the spinal needle is visualized well inside the ventricle, the stylet is removed, and an assistant draws fetal blood into a syringe. A sample of 0.5–3 mL of fetal blood is withdrawn.[6]

Technique of Performing Intrahepatic Portion of Umbilical Vein Sampling

It is considered a less preferable option because it carries a high risk of procedure-related fetal loss (3–4%), it is hence reserved for cases where cordocentesis fails or cannot be performed because of the fetal position. The maternal abdomen is prepared. 20–22 Gauge needle is introduced freehand under ultrasonic guidance into the fetal abdominal wall which is approached either anteriorly near the midline or from the left or right hypochondrium, the needle is then advanced through the substance of the liver parenchyma into the umbilical vein or its craniodorsal continuation, i.e., the left portal vein. The needle is then left unheld. This allows the needle to move freely with fetal movements and minimizes the risk of dislodgment. No sedation is given to the fetus. Once the tip of the spinal needle is visualized well inside the intrahepatic portion of the umbilical vein, the stylet is removed, and an assistant draws fetal blood into a syringe.[7,8]

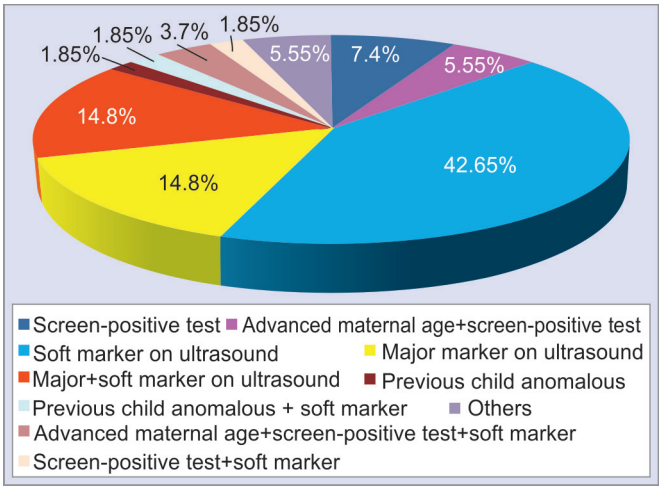

Fig. 1: Indications for fetal blood sampling

Statistical Methods

Descriptive statistical analysis has been carried out in the present study. Results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. *Z*-test for a single proportion has been used to test the significance of study characteristics. 95% confidence intervals have been computed, $p \leq 0.01$ was strongly significant.

The Statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1, and Systat 11.0 were used for the analysis of the data, and Microsoft Word and Excel have been used to generate graphs, tables, etc.

Results

In our study, the average maternal age of patients undergoing fetal blood sampling was 27.51 ± 5.16. Most of the patients (37.03%) were between 21 and 25 years of age-group.

Most of the patients presented at a gestational age of 21–24 weeks (61.15%) as patients usually come for genetic counseling after second-trimester maternal serum screening or after second-trimester fetal anomaly scan.

In total, 20.4% of patients in this study had a consanguineous marriage, out of which 90% of them were second-degree consanguinity.

There were equal number of primigravida (48.15%) and patients belonging to multiparity group (48.15%).

Figure 1 shows that the most common indication for fetal blood sampling in this study was abnormalities detected on ultrasound which accounted for 72.25% of all indications.

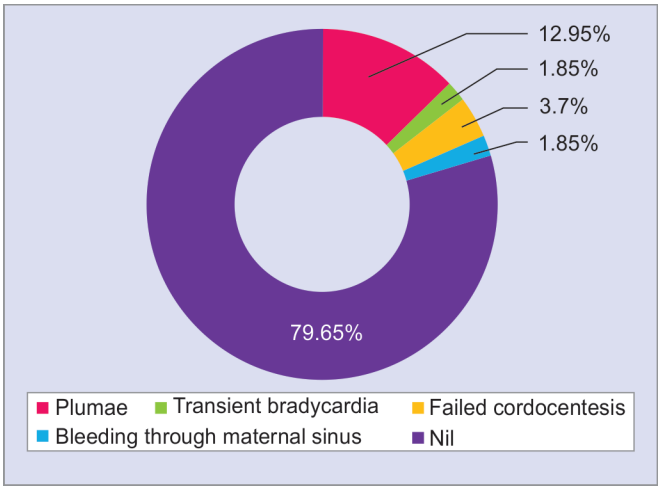

Fig. 2: Complications of the procedure

Table 1: Correlation between placental location and procedure complications

Placental location	*Number (n = 54)*	*Complications*	*%*
Fundal	5	Nil	–
Fundo-anterior	26	6	23.0
Fundo-posterior	17	4	23.5
Anterior wall	5	1	20
Posterior wall	1	Nil	–

The most commonly performed technique of fetal blood sampling in this study was cordocentesis, which was performed on 51 patients (94.45%), out of which two cordocentesis failed while performing and fetal blood could not be obtained, hence amniocentesis was done on these two patients. On one patient, amniocentesis culture failed; hence, cordocentesis was done. Two patients (3.7%) underwent cardiocentesis, one for hydrops fetalis with fundoposterior placenta and the other for fetus with large cystic hygroma with early hydropic changes and coarctation of aorta with fundopanterior placenta. One patient (1.85%) on whom intrahepatic portion of umbilical vein sampling was done had fundoposterior placenta with cortical hyperechogenicity, abnormal sulcation, and intraventricular hemorrhage with traction retinal detachment.

Figure 2 shows that of the cordocentesis procedures performed, on 2 (3.7%) patients, the procedure failed to obtain fetal samples. There was transient bleeding at the puncture site in 7 (12.95%) cases and 95% CI 6.42–24.42, all of them belonged to the cordocentesis group. No immediate complications were noted after cardiocentesis or intrahepatic portion of umbilical vein sampling. No other major complications were noted in this study. In this study, complications (8 out of 33 patients) were most commonly seen at a gestational age of 21–24 weeks, and the patients who underwent the procedure were also more at the same gestational age-group ($n = 33$).

In this study, complications were almost similar in fundo-anterior placenta 23% (6 of 26) and fundo-posterior placenta 23.5% (4 of 17) as shown in Table 1.

Table 2 shows that of the 54 patients who underwent fetal blood sampling, 2 patients on whom cordocentesis failed and

Table 2: Laboratory analysis

Type of test	*Number of patients*	*%*	*95% CI*
Karyotype	51	98.05	84.9–98.1
Kleihauer–Betke test	52	100	87.5–98.9
Complete blood count	4	7.7	2.9–17.6
Infection profile	5	9.6	4.01–19.9
FISH	2	3.85	1.0–12.54
Biochemical analysis	1	1.9	0.3–9.8
Thyroid profile	1	1.9	0.3–9.8
Molecular diagnosis	1	1.9	0.3–9.8

Table 3: Defects detected

Defects	*Number*	*%*
Down's syndrome	3	5.85
Turner's syndrome	1	1.9
Klinefilter's syndrome	1	1.9
Inversions	2	3.85
Translocation Down's	1	1.9
46, add(15)(q25)	1	1.9
Fetal hypothyroidism	1	1.9
G6PD deficiency	1	1.9

amniocentesis was performed were excluded, and the remaining 52 patients were followed up. Karyotype was done on 98.05% (95% CI 84.9–98.1) of the fetal blood samples. On one intrahepatic vein sample of fetal blood, karyotype was not done. Kleihauer–Betke test was performed on all fetal blood samples in order to confirm if the sample obtained was positive for fetal cells. Infection profile was done on 5 (9.6%) patients. Complete blood counts were done on 4 (7.7%) patients. Biochemical analysis was done on 1 patient for Glucose-6-phosphate dehydrogenase deficiency and the indication being Hydrops fetalis. Thyroid profile was done on 1 patient for the indication being fetal thyromegaly.

Molecular diagnosis was done for cystic fibrosis, and the indication being IUGR with echogenic bowel. Fluorescence *in situ* hybridization analysis was done for 2 patients, one for chromosome 21 and the other for 22q deletion.

In 1 patient (1.85%), karyotype culture failed which was on cord blood sample, amniocentesis was done on this patient and the karyotype report was normal. Karyotype was abnormal in 9 patients (17.65%) and 95% CI 63.05–85.36, and at the end of pregnancy, the morphological features of the fetuses were consistent with the abnormal karyotype. Of the 41 patients with normal karyotype, none of the babies had a chromosomal abnormality.

Table 3 shows that the most common defect detected was autosomal trisomy21.

Five out of 8 (62.5%) procedures done in patients with major + soft markers on ultrasound were positive. This correlation was statistically significant (p <0.007). If patient had major and soft markers, the chances of having chromosomal abnormality were more than with other indications. Of the 21 indicated patients with soft markers, one was positive for 45,-14,-15t (14;15) (q10;q10). Apparently Balanced Robertsonian translocation carrier, i.e., 4.75%.

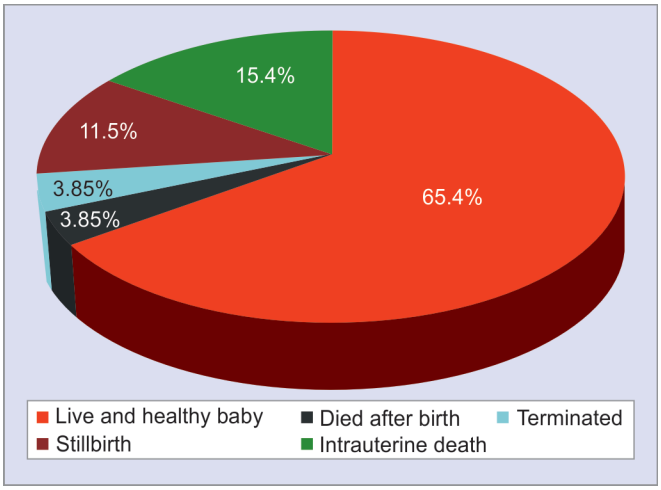

Fig. 3: Outcome of the pregnancy

This had a "*p*" value of 0.124, and of the 8 cases with major markers on ultrasound, none were positive. Of the 2 patients with screen-positive test and with elderly gravida as the indication for procedure along with soft markers on ultrasound, one of the patients was positive for trisomy 21. The "*p*" value was 0.387.

On one patient on whom cordocentesis was done for fetal thyromegaly, the fetus was positive for fetal hypothyroidism and for 46,inv (9) (p11;q13).

Figure 3 shows that of the six patients who had stillbirth, the various causes were 2 patients with Down's syndrome, one patient with glucose-6-phosphate dehydrogenase deficiency who underwent cordocentesis, one patient with early hydrops fetalis with IUGR, esophageal fistula and polyhydramnios, one patient with soft + major marker, 46,add(15)(q25).

One patient who underwent intrahepatic vein portion of umbilical vein sampling for fetus with intraventricular hemorrhage with traction retinal detachment and porencephaly delivered a congenitally malformed baby, baby expired after 6 hours of birth. Another patient, who had left-sided congenital diaphragmatic hernia and liver up position, had preterm delivery and the baby died after 2 hours of birth.

Of the eight intrauterine deaths, the causes were Klinefelter's syndrome, Down's syndrome, 45,-14,-15t (14;15) (q10;q10) apparently balanced Robertsonian translocation, 46, inv(2) (p12;q21), 46, inv (9) (p11;q13), fetal hepatomegaly, bladder outlet obstruction with B/L hydroureter and duodenal atresia and one patient with severe IUGR, lissencephaly, and cerebellar hypoplasia. The normal live and healthy baby rate of our study was 65.5% (95% CI 51.80–76.9).

The termination rate was 3.85% (CI 1.06–12.98). Indications were Turner's syndrome and malascended right kidney with cystic dysplasia of left kidney with delayed fetal nasal bone ossification and single umbilical artery.

Discussion

This study consists of 54 patients who underwent fetal blood sampling of the patients who came for counseling and procedure during the study period at this center after fulfillment of inclusion and exclusion criteria. The mean maternal age in this study was 27.15 years and presented mostly in gestational age between 23.83 weeks because the patients usually came for genetic counseling after second-trimester screening by maternal serum biochemical testing or by second-trimester fetal anomaly scan unlike the study done by Tongsong et al.,[7–10] in which the mean maternal age was 31.1 years and the mean gestational age at the time of cordocentesis was 19.8 weeks. Consanguinity which was present in 20.4% of cases in this study plays a major role in diseases leading to mortality of the progeny as a consequence of detrimental recessive genes. However, it is not an indication for prenatal diagnostic testing.

At this center, cordocentesis was the procedure of choice for fetal blood sampling, it was performed on 94.4% ($n = 51$) patients, similar to the study carried out by Haddow et al.[11] in which cordocentesis was done on 93.3% of patients.

Karyotype was done on 51 samples of fetal blood, 17.65% had karyotype abnormalities, and cord blood culture failure in 1.85%, all samples obtained were positive for fetal cells, and no samples were obtained inadequately in this study, unlike the study done by Wald et al.[12] in which karyotype abnormalities were noted in 5.2%, maternal contamination of the sample in 12.3%, inadequate sample in 6.4%, and culture failure in 9.8%.

Advanced molecular and biochemical techniques proved useful in 23% of cases which included inborn errors of metabolism, serology for viral infections, complete blood count, and thyroid profile. Fluorescence *in situ* hybridization analysis was done in 3.85% (2 patients).

Of the patients with screen-positive tests and with elderly gravida as the indication for the procedure, none of the patients were detected to have any cytogenetic abnormality. This was unlike other studies according to which triple test has a high sensitivity. This may be due to the small sample size. Moreover, in this center, patients were referred on whom the triple test was done in different centers, among which multiples of median and risk estimates had a wide variation. This was similar to the Korean lab study which concluded that an external quality control and standardization of the variable seem to be warranted.[13,14]

The pregnancy termination rate in this study was 3.85% ($n = 2$), and indications for termination were Turner's syndrome and malascended right kidney with cystic dysplasia of left kidney with delayed fetal nasal bone ossification and single umbilical artery.

Out of 96.15% ($n = 50$) who continued their pregnancy, 65.4% ($n = 34$) had normal live and healthy babies.

Conclusion

Fetal blood sampling is a safe and effective prenatal diagnostic procedure in experienced hands, mostly performed at 21–24 weeks of gestation. Cordocentesis can be done as an alternative to amniocentesis after 18 weeks of pregnancy as results of karyotype can be obtained earlier. In such cases, FISH can be avoided to cut down the cost.[15] The risk of culture failure and fetal loss is also acceptable. Methods like fetal blood sampling, which makes karyotyping possible within a short time, should be preferred to amniocentesis and FISH analysis because FISH does not detect chromosomal mosaicism and structural aberrations. Triple test screening is on an increase, but it requires an external quality control program and standardization of the variables. Soft markers of ultrasound are a reliable screening test. Major anomalies on ultrasound are likely to be associated with cytogenetic abnormality. Hence, these fetuses need a cytogenetic study preferably before termination. This would aid in counseling for future pregnancies. Cardiocentesis or intrahepatic portion of umbilical vein sampling may be used as alternate method when cordocentesis is difficult to perform. Complications were similar in both fundo-anterior and

fundo-posterior placental locations. Although this study consists of 54 patients on whom fetal blood sampling was done, larger studies are needed to validate the conclusions of this study.

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