CASE SERIES

Six-year experience of prenatal diagnosis for beta thalassemia in twin pregnancies and selective foetal reduction — A case series

Yasmeen Ehsan[1], Shabnam Bashir[2], Furqan Sabir[3], Mahmood Ghafoor[4], Shagufta Khaliq[5]

Abstract

A retrospective study was conducted for prenatal diagnosis and selective foetal reduction, which included 41 beta thalassemia carrier couples with twin pregnancy. Chorionic villi sampling was carried out at 12-14 weeks of gestation. Thirty-three couples presented with dizygotic while eight had monozygotic twins. Molecular analysis for beta thalassemia revealed similar results in both the twins in 27 cases, while different results were obtained in 14 cases. Selective foetal reduction was offered to couples with discordant results. One miscarriage occurred due to the procedure. The results of prenatal molecular analysis were confirmed by postnatal molecular analysis. This series of trans-abdominal Chorionic villi sampling in twin pregnancies with the option of selective foetal reduction of the affected foetus was found to be useful as well as acceptable by the at-risk couples.

Keywords: Chorionic Villi, Prenatal diagnosis, Genetic Counseling, Beta-Thalassemia, Selective Foetal Termination

DOI: https://doi.org/10.47391/JPMA.716

Introduction

Beta thalassemia is an autosomal, inherited haemoglobin disorder with 1.5% prevalence rate worldwide.[1] It is the most common inherited disorder in Pakistan with a carrier rate of 5.0% and about 6,000 Beta Thalassemia major (BTM) children born per year.[2] Chorionic Villi Sampling (CVS) has been the widely accepted technique for beta thalassemia genetic testing with the advantage to diagnose the affected foetus in the first trimester of pregnancy.[3]

Sometimes at-risk couples present with twin pregnancies and prenatal diagnosis is more complicated in these cases. It is important that twin pregnancy is identified as early as possible. Visualisation of gestational sacs at 35th day of menstrual cycle is possible with trans-vaginal ultrasound and cardiac activity can be confirmed on 44th day of the cycle.[4] Twin pregnancies are dated between 11-13+6 weeks of gestation (i.e. crown rump length is 45-84mm) Grade of recommendation D.

Documentation of amnionicity and chronicity is important to define the zygosity of twin pregnancies.[5,6] Chronicity is determined by the insertion of amniotic membrane into the placenta.[7] This can be assessed by noting the T sign or λ sign and the number of placental masses.[8] The T sign is seen in monochorionic twins while the λ sign (figure 1) is seen in dichorionic twins. Monochorionic twins form 20% of all twin pregnancies and share a single placenta,[9] whereas dizygotic, dichorionic twins are always di-chorionic-diamniotic with two separate placentas. In case of dichorionic twins, it is recommended to label and document the position so that if a foetus is found to be affected then selective termination can be carried out safely. Moreover, selection of different placental sites for sample collection in cases with dichorionic twins has been recommended for reliable genetic results for prenatal diagnosis.

Prenatal genetic counselling is provided to at-risk couples with twin gestations with the option of selective termination of the affected foetus. This is carried out by intra-cardiac or intra-cranial injection of 2-3ml of 15% potassium chloride.[10] The present study reports our experience of 41 CVS cases in twin pregnancies at the Punjab Thalassemia Prevention Project.

Subjects and Methods

The study was conducted retrospectively on 41 couples from 2012 to March 2019 at the Punjab Thalassemia Prevention Programme, Sir Ganga Ram Hospital, Lahore. Non-directive genetic counselling was provided to all such couples and written consent was obtained for inclusion in the study. Gestational age, foetal number and viability with positioning of placentas were evaluated and discussed.6

CVS procedures were carried out at 12-14 weeks of gestation after a detailed ultrasound to establish the zygosity, chronicity and viability of foetuses. Single sample was obtained in monozygotic twins, while double samples were obtained in dizygotic twins by trans-abdominal route under ultrasound guidance. Blood samples of parents were collected in EDTA vials. DNA was extracted from blood and Chorionic villi tissue by GF-1 DNA Extraction Kit (Vivantis).

[1,3]Punjab Thalassemia Prevetion Program (PTPP), Lahore, Pakistan; [2]Department of Pathology, Punjab Thalassemia Prevention Program, Fatima Jinnah Medical University, Lahore, Pakistan; [4]Department of Molecular Biology, Punjab Thalassemia Prevetion Program (PTPP), Lahore Pakistan; [5]Department of Human Genetics and Molecular Biology, University of Health Sciences Lahore, Pakistan

Correspondence: Shabnam Bashir. e-mail: docshabnam2000@yahoo.com

Mutation analysis was carried out by ARMS-PCR using allele specific primers for 12 frequently reported β thalassemia mutation from Pakistan.

A post aspiration ultrasound was done to ensure the foetal viability and to rule out any retro placental haematoma formation or placental separation. Zygosity and chronicity of the twins was confirmed by a detailed ultrasound for applying single or double entry needle approach. Figure 1 shows the T and λ signs for the mono and dizygosity, respectively, and the intra-cardiac needle for selective foetal reduction. Selective foetal reduction was carried out for pregnancies with discordant results, i.e. one foetus was homozygous BTM while the other was normal or heterozygous carrier. Foetal reduction was achieved by intra-cardiac injection of 2 ml of 15% KCl solution. To monitor the cardiac arrest, Asystole was noted within two to three minutes. Repeat ultrasound was performed after one hour to confirm the demise of the selected foetus.

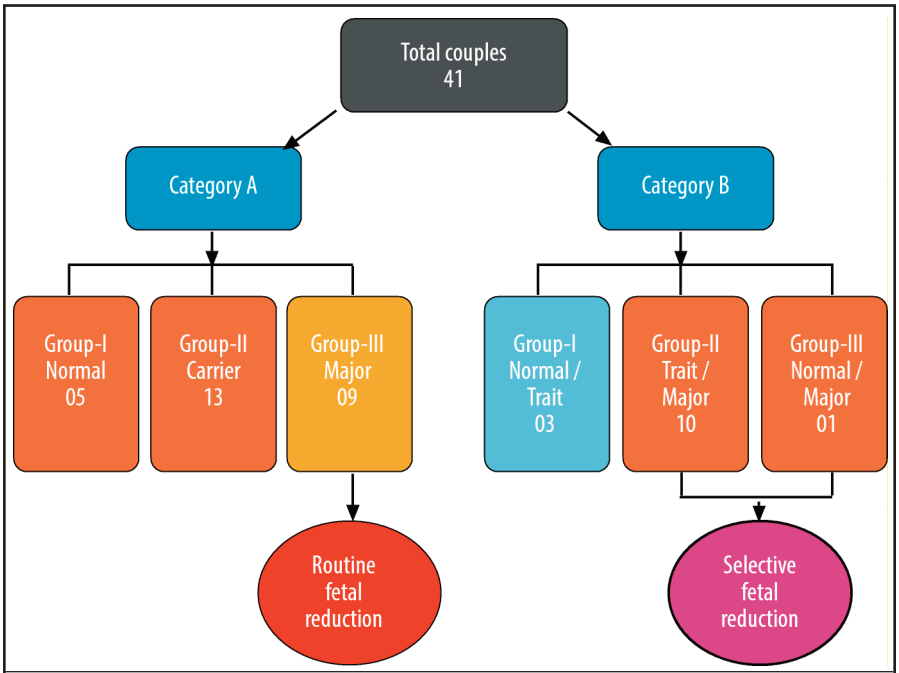

Figure-1: Illustration of the study protocol.

Complete follow up was obtained in all cases and information about the newborn's phenotype was collected 12 months after the expected date of birth.

Results

Out of the 41 couples, mono-zygosity was found in eight cases while di-zygosity was observed in 33 cases.

Mutation analysis through ARMS-PCR revealed Fr 8-9, IVS 1-5, Fr 41-42, Fr-16, Del-619, Cd-5, Cd-15, CAP+1, Hb-E and Cd-30 mutations. In this study, the most commonly found mutations were Fr 8-9 and IVS 1-5 followed by less common mutations.

The mutation analysis results of 41 couples fall into two categories as described in figure 1.

In the first category, similar results were found in 27 cases, i.e., in 5 cases neither of the foetus had mutation (normal), in 13 cases both of the foetuses were heterozygous or carrier of beta thalassemia, while in 9 cases both the foetuses were homozygous for beta thalassemia (major). The routine termination procedure was followed for these 9 cases.

In the second category, discordant results were found in 14 cases and were divided into three groups. Out of these, group I (03 cases) reported one foetus as normal and the other as beta thalassemia carrier.

In group II (10 cases), one foetus was found as beta thalassemia carrier but the second foetus as BTM.

In group III (01 case), one foetus was normal while the other was BTM.

Obliviously, for the last two groups (11 cases) from the second category, with conflicting results, option of selective termination was desired. These couples were offered selective foetal reduction after genetic counselling.

Seven 07 couples agreed for selective termination and foetal reduction was carried out successfully by intra-cardiac injection in five cases and intra-cranial injection in two cases.

Selective termination was not possible, unfortunately, in four cases. The fear of loss of the second twin did not allow one mother to accept this procedure, while the remaining three cases took long to decide and exceeded the time limit of 17 weeks (beyond 17 weeks, foetal reduction is prohibited according to Islamic fatwa).

In this study, foetal loss due to CVS was reported in only one case (2.43%). After excluding nine terminated pregnancies of homozygous foetuses, one miscarriage due to CVS, the postnatal outcome in 31 pregnancies was in line with our prenatal diagnosis.

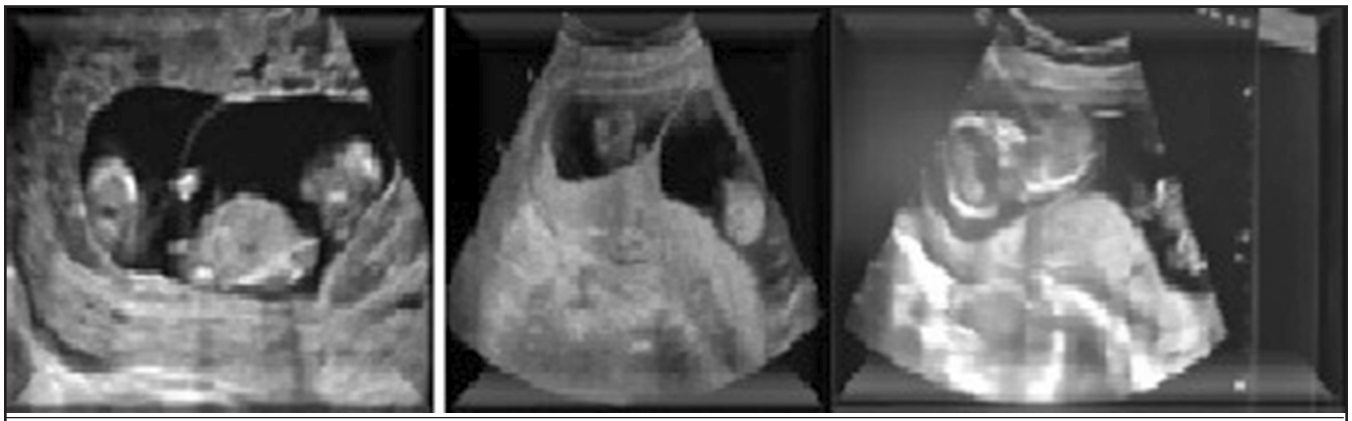

Figure-2: Mono and Dizygotic Twins with T & λ Sign respectively and Intra cardiac injection for selective foetal reduction.

Discussion

Among the at-risk couples, the possibility of having a child with thalassemia major is doubled in twin gestations. The situation becomes more complicated when the genetic analysis reveals one twin normal or heterozygous (carrier) and another twin homozygous (affected). The prenatal diagnosis for such cases requires more accuracy than in singleton gestation. Feasibility and risks of prenatal diagnosis by CVS in twin pregnancies have been evaluated in several studies. To our knowledge this is the first report of its kind from Pakistan.

A total of 41 at-risk couples were included in the present study. Out of these, 18 pregnancies carried monozygotic twins while 32 pregnancies had dizygotic twins. The most common mutations were found to be IVS1-5 and Fr8-9 through molecular characterisation of beta globin gene of foetal tissue, that is in accordance with previous studies.[11] Upon DNA analysis of CVS tissue, two categories were reflected for dizygotic twins (figure 2).

Category A exhibited both the twin foetuses with the same status, i.e. both the twin were normal, trait or homozygous for beta thalassemia. BTM cases from this category did not require selective termination, and rather followed the conventional procedure for foetal reduction.

Category B comprised those couples where twin foetuses carried discordant status as shown in figure 1. Selective foetal reduction was required only in those cases where one of the foetuses was homozygous for beta thalassaemia, while the other foetus was either normal or trait. In 11 cases selective foetal reduction of BTM foetus was desired.

After extensive genetic counselling sessions with the concerned couples, 07 of them agreed for selective foetal reduction.

In 05 cases, the process of foetal reduction was carried out by injecting 15 % KCL intra-cardically precisely into the affected foetus. In 02 cases, because of rapid foetal movement, intra-cranial injection was used to ensure that the unaffected foetus is not harmed. Both of these methods were found to be successful for selective foetal reduction and has been used in some other studies.[10,12] Four couples did not exercise this choice to selectively abort the affected foetus. One couple rejected the option straightaway due to fear of losing the unaffected baby due to the procedure.

The other 03 couples were reluctant in making a timely decision for the procedure and exceeded the time limit of 17 weeks. According to Islamic fatwa regarding foetal reduction, the ensoulment of the foetus occurs at 120th day of gestation and pregnancy cannot be terminated after this time limit. Hence, a timely decision regarding reduction of the affected foetal must be made by the families to avoid any undesired circumstances. Again, the major hindrance in acceptance of this choice was the above mentioned reason as well as other social and religious factors present in our society that make it difficult for these at-risk couples to opt for the option of abortion of the affected foetus.

A complete follow up was obtained in all cases and information on new-born phenotype was collected after the expected date of birth. It revealed that all the cases of selective foetal reduction were correctly employed and no misdiagnosis was found due to the procedure. Previously, reported rate of loss of the second foetus after selective foetal reduction was 10%,[13] but in this study no loss of the second twin occurred. This shows the effectiveness, and efficiency of this method.

Conclusion

This study gives novel information about the prenatal diagnosis of β thalassemia in twin gestations and provides reassuring evidence regarding the risk of pregnancy loss

associated with CVS procedure and selective foetal reduction.

Early diagnosis also helps in selective termination of the affected foetus at an early stage and is associated with higher survival rate of the second twin. This provides reassurance of foetal wellbeing and, therefore, reduces parental anxiety and uncertainty.

Disclaimer: None.

Conflict of interest: None

Funding disclosure: The project was funded by the HealthDepartment, Government of Punjab, Pakistan

References

1. Hossain MS, Raheem E, Sultana TA, Ferdous S, Nahar N, Islam S, et al. Thalassemias in South Asia: clinical lessons learnt from Bangladesh. Orphanet J Rare Dis 2017; 12: 93.
2. Ikram N. Role of prenatal diagnosis in thalassaemia prevention. JRMC 2017; 21: 109-12.
3. Choudry A, Masood S, Ahmed S. Feasibility and safety of transabdominal chorionic villus sampling. J Ayub Med Coll Abbottabad 2012; 24: 38-43.
4. Monni G, Iuculano A, Zoppi M. Screening and invasive testing in twins. J Clin Med 2014; 3: 865-82.
5. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics; Committee on Genetics; Society for Maternal–Fetal Medicine. Practice Bulletin No. 162: Prenatal Diagnostic Testing for Genetic Disorders. Obstet Gynecol 2016; 127: e108-22.
6. Audibert F, Gagnon A. Prenatal screening for and diagnosis of aneuploidy in twin pregnancies. J Obstet Gynaecol Can 2011; 33: 754-67.
7. Khalil A, Rodgers M, Baschat A, Bhide A, Gratacos E, Hecher K, et al. ISUOG Practice Guidelines: role of ultrasound in twin pregnancy. Ultrasound Obstet Gynaecol 2016; 47: 247-63.
8. Li DZ, Pan M, Han J, Yang X, Zhen L, Li J, et al. Prenatal diagnosis of thalassaemia in twin pregnancies in mainland China. J Obstet Gynaecol 2016; 36: 731-4.
9. Fichera A, Prefumo F, Stagnati V, Marella D, Valcamonico A, Frusca T. Outcome of monochorionic diamniotic twin pregnancies followed at a single center. Prenat Diagn 2015;35:1057-64.
10. Li R, Yang R, Chen X, Yang S, Ma C, Liu P, et al. Intracranial KCl injection-an alternative method for multifetal pregnancy reduction in the early second trimester. Fetal Diag Ther 2013; 34: 26-30.
11. Baig S, Azhar A, Hassan H, Baig J, Kiyani A, Hameed U, et al. Spectrum of beta-thalassaemia mutations in various regions of Punjab and Islamabad, Pakistan: establishment of prenatal diagnosis. Haematologica 2006; 91: ELT02.
12. Govender L. Techniques of Fetal Reduction and Feticide. South Afr J Obstet Gynaecol 2010; 16: 38-40.
13. De Catte L, Foulon W. Obstetric outcome after foetal reduction to singleton pregnancies. Prenat Diag 2002; 22: 206-10.