Original Article

Prenatal Diagnosis of Beta-Thalassemia Disease and Fate of Fetuses Among Beta-Thalassemia Carrier Mothers: An Experience at Tertiary Care Hospital of Rahim Yar Khan

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ABSTRACT

Objective: To assess the status of beta thalassemia disease in fetuses of beta thalassemia carrier mothers and to determine the fate of these fetuses.

Study Design: A cross sectional study

Place and Duration of Study: This study was conducted at the Pathology and Gynecology & Obstetrics Departments of Sheikh Zayed Medical College/Hospital, Rahim Yar Khan from July 2022 to June 2023.

Materials and Methods: Ninety pregnant women with known thalassemia carrier status participated in the current study. Beta-thalassemia carrier status was confirmed at Pathology Department while antenatal examination and assessment was performed at Department of Gynecology & Obstetrics. After getting the DNA and PCR done, the fate of fetuses was recorded on follow up of the study subjects. The collected data was analyzed using version 25.

Results: Of the 90 women who had CVS, 21 (23%) had fetuses with the thalassemia major gene, 34 (37%) had fetuses with the thalassemia minor gene, and 26 (28%) had fetuses that were otherwise healthy and normal. Nine (10%) women experienced miscarriages either before or after the test. Thirteen (62%) of the 21 patients with fetuses of thalassemia major gene underwent pregnancy termination.

Conclusion: The results of the current study showed that a significant number of mothers carry fetuses with thalassemia major gene which strongly imply that prenatal diagnosis of thalassemia major and CVS aid in prompt decision-making regarding pregnancy termination in thalassemia major, hence reducing the disease burden.

Key Words: Beta-Thalassemia, Carrier, Chorionic Villus Sampling (CVS), Prenatal Diagnosis, Fetal outcome

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INTRODUCTION

Hemoglobinopathies are inherited disorders affecting more than 300,000 newborns every year. Thalassemia is the most prevalent hemoglobinopathy in more than 60 countries as reported by World Health Organization.[1] Adult hemoglobin constitutes four globin chains, two alpha and two beta with different amino-acid sequences, revealing hemoglobin A (α2β2).

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In thalassemia disease, insufficient globin chain synthesizes, which results in the generation of defective hemoglobin. There are three different forms of this condition: thalassemia major, thalassemia intermediate, and thalassemia minor. TDT (transfusion-dependent beta thalassemia) and non-TDT (non-transfusion-dependent beta thalassemia) are the two clinical subtypes of thalassemia. More than 26,000 patients with transfusion-dependent thalassemia are born every year; 90% of these births occur in developing and middle-income countries.[2, 3] Estimated 200 mutated beta globin genes have been identified globally which cause β-thalassemia disease. When two individuals carrying heterozygous status get marry, the risk of the birth of thalassemia major child will be 25% in every pregnancy. It is appraised that 1.5% of the total population around globe is beta thalassemia carrier. β-thalassemia major is the common genetic disorder in Pakistan with 40,000 transfusion dependent children, while beta thalassemia carrier individuals are 5-7% (nine million). Five thousand to nine thousand children with thalassemia major are born each year and require

regular blood transfusions to survive.[4] The asymptomatic β-thalassemia carriers with a single β-globin mutation present with microcytosis and mild anemia. Thalassemia intermedia patients do not require frequent transfusions in comparison to beta thalassemia major patients who suffer from hypochromic microcytic anemia with severe hemolysis. The erythrocytic degradation and the need of frequent blood transfusions in thalassemia major patients results in iron accumulation inside essential organs specifically in heart, spleen, liver, pancreas and endocrine glands. These individuals require constant iron chelation therapy in addition to lifelong blood transfusions to manage their anemia and the iron excess. Patients with untreated thalassemia face secondary problems, such as cirrhosis, low muscle tone, pallor, jaundice, leg ulcers, and hepatosplenomegaly.[5, 6]

The only prevention strategy for this severe genetic disease discovered after many decades is the identification of the genetic status and in time elimination of afflicted fetuses. This can only be accomplished through targeted premarital screening of thalassemic families and prenatal thalassemia diagnosis. Although the provincial assemblies of Punjab, Sindh, and Khyber Pakhtunkhwa had passed the pre-marital thalassemia screening bill, it was sadly not put into effect. Due to the religious beliefs, the exorbitant expense of the tests, and the lack of genetic counselling, only a small number of couples choose this option in Pakistan. Cell-free foetal DNA (cffDNA) is obtained from maternal plasma and fetal cells during chorionic villus sampling (CVS) at 10–12 weeks of gestation for prenatal diagnosis by DNA testing.[7] Since 1980, CVS has been used to quickly diagnose genetic abnormalities during pregnancy. The year 1994 saw the first prenatal testing for thalassemia in Pakistan.[8] This traumatic technique may cause injury to the fetus, miscarriage, intrauterine infection, preterm delivery or fetal limbs deformities. Additional disadvantage of CVS include challenging cytogenetic analysis, contamination by maternal cells and the chance of mosacism.[9]

According to a fatwa issued by Mufti Taqi Usmani, a well-known religious figure in Pakistan, an abortion may be conducted between 120 days after conception but not later than that, CVS is permissible in the religion of Islam. Through CVS, antenatal thalassemia diagnosis can be performed, and appropriate actions can be taken to lessen the burden on society.

This study is the first of its kind to be planned with objective to examine the status of beta thalassemia disease in fetuses of beta thalassemia carrier moms and to determine the fate of these fetuses by CVS.

MATERIALS AND METHODS

This cross-sectional was conducted from July 2022 to June 2023. Carrier status of the pregnant mothers and their husbands was diagnosed in Pathology Department by Complete Blood Count, Peripheral Blood Morphology and HB Electrophoresis while ante-natal examination and preparation for CVS was done at Department of Gynecology & Obstetrics, Sheikh Zayed Medical College/Hospital, RahimYar Khan. A sample size of 88 was calculated, rounded to 90, with an expected proportion of normal delivery of 66%, 95% confidence interval and 5% margin of error. Convenient sampling technique was utilized. As the facility of CVS and DNA analysis was not available in Rahim Yar Khan during the study period, the selected mothers were referred to the centers located in Bahawalpur and Lahore where these services were provided free of cost. The fate of fetuses was recorded on follow up of the study subjects. The collected data was analyzed using Statistical Package for Social Science (SPSS) 25. Ethical approval for study conduction was got from Institutional Review Board of Sheikh Zayed Medical College/Hospital, Rahim Yar Khan. Informed verbal consent was taken from every study subject.

Chorionic Villous Sampling (CVS) is a prenatal diagnostic procedure that can be used to detect thalassemia mutations. During CVS, a small sample of placental tissue is obtained using a thin needle inserted through the mother's abdomen and into the uterus. The placental tissue sample is then analyzed in a laboratory using polymerase chain reaction (PCR) to detect specific thalassemia mutations in the fetal DNA.[10, 11]

RESULTS

A total 90 beta-thalassemia carrier mothers were included in the current study. The mean age of study subjects was 28±5 years. Area wise distribution of the study subjects showed 50(55.5%) mothers from rural areas and 40 (44.4%) belonging to the urban areas. Out of these 90 mothers, 65 (72.2%) were married to their cousins or close relatives. Descriptive statistics of gestational age estimated through ultrasound showed the mean gestational age of fetuses as 11.5±2.28 weeks. Table-1 shows the diagnosis made by CVS analysis. The results showed that beta-thalassemia major were 21(23%), beta-thalassemia trait were 34(37%), normal fetuses were 26(28%) and 9(10%) had miscarriage before or after the CVS procedure. The fate of fetuses after CVS has been shown in Figure-1. As mentioned earlier, out of 90 study subjects, 21 fetuses (23%) had been diagnosed as thalassemia major, 13(61%) mothers out of these 21, decided to terminate their pregnancies. Rest fetuses faced different fates, 9(10%) had miscarriage, 3 were stillborn (3.3%), 22(24.4%) were anemic at the time of birth and 26(28%) were apparently normal at the time of birth. Table-2 showed percentage of consanguinity among couples in Urban and Rural areas. Overall consanguinity was seen in 65 patients (72.2%). Consanguinity is seen more prominently in rural areas (42.2%) than in urban areas (30%)of total patients (p value =0.3). Table-Ishowed

area frequency of diagnosis through CVS according to residence area.

Table No. 1: Overall and Residence wise frequency distribution of Diagnosis

Residence	Diagnosis					P value
	Thalassemia Major	Thalassemia Trait	Normal fetus	Miscarriage before/after CVS	Total	
Urban	7 (17.5%)	19 (47.5%)	10 (25%)	4 (10%)	40 (44.4%)	**0.35**
Rural	14 (28%)	15 (30%)	16 (32%)	5 (10%)	50 (55.6%)	
Total	21 (23.3%)	34 (37.7%)	26 (28.8%)	9 (10%)	90 (100%)	

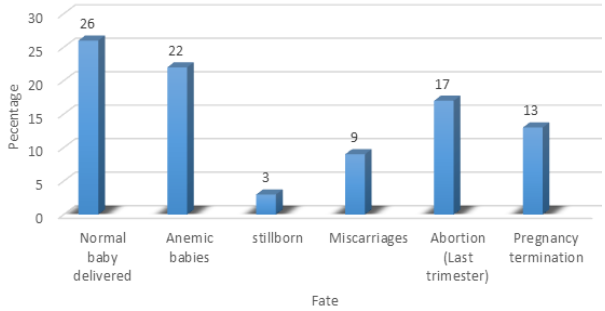

Figure No. 1: Fate of Fetuses after Chorionic Villus Sampling

Table No. 2: Area wise distribution of Consanguinity among couples

Residence	Consanguinity		Total	P value
	Yes	No		
Urban	27 (30%)	13 (14.4%)	40 (44.4%)	0.3
Rural	38 (42.2%)	12 (13.3%)	50 (55.6%)	
Total	65 (72.2%)	25 (27.8%)	90 (100%)	

DISCUSSION

Iron chelating therapy as well as blood transfusions are part of the standard treatment for Beta thalassemia disease to control the deleterious effects of progressive iron overload.[12] In individuals without signs of iron overload, bone marrow donation from HLA-identical siblings leads to disease-free survival and has considerably improved not just the survival but also the quality of life for thalassemia patients.[13] However, these modes of management are typically not widely accessible in developing countries where thalassemia is common and health care resources are scarce. Therefore, prevention like premarital screening, carrier screening, prenatal diagnosis is the best and most economical strategy for solving this issue. This study was done to check the status of fetuses with thalassemia carrier mothers. Out of 90 couples who went for CVS, 21 (23%) were expecting fetuses with thalassemia major gene mutation, 34 (37%) were pregnant with fetuses who had thalassemia trait and 26 (28%) were diagnosed to have normal fetuses. In this study 10 (11%) patients out of 90 had miscarriages before or after the CVS. Similar study results were observed in Islamabad, where out of 28 cases of CVS, 5 went for termination of pregnancy with thalassemia major diagnosis and 9 were thalassemia minor patients[14]. In another study from Iran among 300 women, 18% fetuses diagnosed as thalassemia major and spontaneous abortion rate was 1.4%. Twelve (20%) of the sixty fetuses in a study from Multan on DNA analysis of post-CVS samples revealed homozygous thalassemia, and all the couples who participated in it chose to abort their fetuses. 1.3-3% fetal loss after CVS have been reported.[15] These study results are in accordance with the present study where 13 out of 21 cases of thalassemia major decided to terminate pregnancies, while miscarriages were seen in total 9 patients. A research conducted in Eastern India showed that out of 235 fetuses 110 (46.8%) fetuses were normal while 20/235 (8.51%) were diagnosed as thalassemia major. Further 70/235 (29.78%) were diagnosed as trait and 5% showed other mutations of globin chains gene.[16] Another study on the geographic distribution and safety of CVS revealed that out of 223 CVS cases (43%) were thalassemia minor, (38%) were thalassemia major and (19%) were normal. The pregnancy loss rate after CVS was 2%.[17] A Chinese study on prenatal testing reported that out of 2116 studied subjects, 315 were healthy fetuses, 500 were thalassemia carriers and 253 were fetuses with beta thalassemia major.[18]

CONCLUSION

The findings of the present study demonstrated that a significant proportion of thalassemia carrier mothers carry fetuses with the thalassemia major gene, which strongly suggests that prenatal diagnosis of thalassemia major and CVS aid in prompt decision-making regarding pregnancy termination, thereby reducing the disease burden. Prenatal diagnosis of thalassemia mutations is imperative so that parents can make educated choices regarding the pregnancy and the care of the new born. Chorionic villous sampling (CVS) is a prenatal diagnostic procedure that can be used to detect thalassemia mutations in fetuses of thalassemia carrier mothers.

Author's Contribution:

Concept & Design of Study:	Muhammad Bilal Ghafoor
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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