

Effectiveness and feasibility of transabdominal chorionic villous sampling procedure for prenatal diagnosis of β -thalassaemia in a Muslim majority community of Pakistan

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ABSTRACT

Objectives: To determine the effectiveness and feasibility of transabdominal chorionic villi sample (CVS) procedure for prenatal diagnosis of β -thalassaemia in a Muslim majority community.

Methodology: Between January 2005 and December 2011, we analysed 798 high-risk mothers with 12-16 weeks of pregnancy for β -thalassaemia using CVS, performed with a transabdominal route under local anesthesia and ultrasound guidance. The chorionic villi extracted were investigated upon using genomic amplification of β -globin gene by polymerase chain reaction (PCR).

Results: A total of 798 of which 224(28%) fetus were diagnosed as major, 400(50.1%) as minor, 173(21.6%) as healthy fetus and 1(0.12%) fetus had undetected mutation. Procedure related complications were seen in 20 cases (2.4%) and missed abortion occurred in 6/798. Seven (3%) couples had refused to abort β -thalassaemia major fetus where as 97% fetus was aborted as per recommendations.

Conclusion: Ultrasound guided transabdominal CVS is an effective procedure for prenatal diagnosis of β -thalassaemia in a Muslim community. We found no cultural hurdles for fetal sampling and prenatal diagnosis.

KEY WORDS: β -thalassaemia, Prenatal diagnosis, Chorionic villus sampling.

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INTRODUCTION

Thalassaemia is a heterogeneous autosomal recessive disorder in which production of normal hemoglobin is partly or completely suppressed because of diminished synthesis of one or more globin chains.¹ The clinically important ones are α and β -thalassaemia. β -thalassaemia is one of the most common monogenic disorder worldwide.² It is also one of the most common or highest prevalent inheritable genetic disorders in Pakistan. The approximate prevalence of β -thalassaemia trait in Pakistan is around 5% and there is increase in burden of 5000 new cases every year in the existing pool.³ β -thalassaemia may exhaust existing health resources and there is a threat that it will become a

major health issue in Pakistan as decline is observed in infection related deaths in children.² Prevention is the only way to address the issue. This can be done through carrier screening, genetic counselling and prenatal diagnosis to control affected birth.⁴

In Pakistan where around ten million people are carrier of β -thalassaemia there should be serious efforts to increase public awareness in order to encourage them to get screened for thalassaemia trait. If both parents are thalassaemia carrier they must undergo prenatal diagnosis through CVS.³ At present the main method of prenatal diagnosis available in Pakistan is CVS for the diagnosis of fetal cytogenetic, molecular and biochemical disorders.⁵ It was initially used as first trimester diagnostic procedure in China by Anguo et al in 1970. It was later introduced in Italy by Brambati and Simoni in 1983 and in Pakistan by Ahmad et al in 1994. However in Pakistan CVS has been shown to be a safe and effective tool for prenatal diagnosis and subsequent counselling in selected couples.⁶⁻⁸

As far as the religious point of view is concerned, there has been many improvements by the religious scholars. The misunderstandings of the Islamic laws have been removed & clarifications have been provided by many of the top level Muslim jurists. According to many fatawa, if continuation of pregnancy becomes life threatening then abortion can be carried out within 120 days of the pregnancy but no later than that.^{9,10}

The aim of this study was to determine the effectiveness and feasibility of transabdominal CVS procedure for prenatal diagnosis of β -thalassaemia in Muslim majority community.

METHODOLOGY

This descriptive study was conducted in the National Institute of Blood Disease (NIBD) and Bone Marrow Transplantation and the Omair Sana Foundation, over a period of six years from January 2005 to December 2011. We analyzed 798 high risk mothers for β -thalassaemia. The studied population included members of five major ethnic groups in Karachi: (1) Balochi, (2) Sindhi, (3) Punjabi, (4) Pathan and (5) immigrants from India (after the 1947 partition of sub-continent). The fifth group also included others like Saraikees, Hazara, Hindko, Memon and Persian. Pregnant women at 12-16 weeks of gestation who fulfilled any one of the following criteria were included in the study: (1) couples with children suffering from β -thalassaemia; or (2) couples who were known carriers for β -thalassaemia.

Women with gestation more than sixteen weeks,

or who refused termination in case the fetus was homozygous for β -thalassaemia were excluded from the study. Before going through the procedure, all couples were interviewed in detail particularly about the number of children affected, ethnic origin, cast, consanguinity and death of an affected child. Subsequently they were counseled about the pattern of inheritance, the chances of having an affected child in the current or future pregnancy, the procedure of CVS, risk and complications of the procedure and the option of termination of pregnancy in case the fetus was detected to be homozygous for β -thalassaemia. Before the procedure an informed consent was obtained from both partners. Once they agreed, all of the women included in the study had a detailed ultrasound scanning for gestational age, viability of fetus and placental localization. The ethical issues related to prenatal diagnosis and a possible termination of pregnancy to follow (Petroiu. 2003), were discussed with the couples in the light of a religious verdict (fatwa).¹¹ After obtaining consent and using all necessary aseptic techniques CVS was done using a trans-abdominal approach under local anesthesia and ultrasound guidance. A Co-axial Chorion Biopsy needle set with an outer guide and an inner aspiration needle was used. The needle was introduced into the placenta in its longitudinal direction. Once the needle was adequately placed, the chorionic villi were aspirated by agitation of the aspiration needle and by applying suction force through a syringe. The placenta was approached in almost any position using trans-abdominal technique without significant risk to the fetus or mother.¹²

The sample was saved in sterilized test tube in normal saline and shifted to molecular lab. The chorionic villi extracted were investigated upon using genomic amplification of β -globin gene by polymerase chain reaction (PCR). Amplification Refractory Mutation System (ARMS) was used for this purpose on a Thermal Cycler (Haier). Genotypes of the patients were analyzed at the Molecular Laboratory, NIBD. After the collection of results, if the fetus was positive for thalassaemia major then the facility for termination was provided. A record of all the pregnancies was maintained. The possible complications of performing the procedure including but not limited to, vaginal bleeding, vaginal leaking, uterine cramps and chorioamnionitis were recorded. The post-procedural miscarriage rate was defined as spontaneous abortion, intra-uterine demise before 24 weeks of gestation.

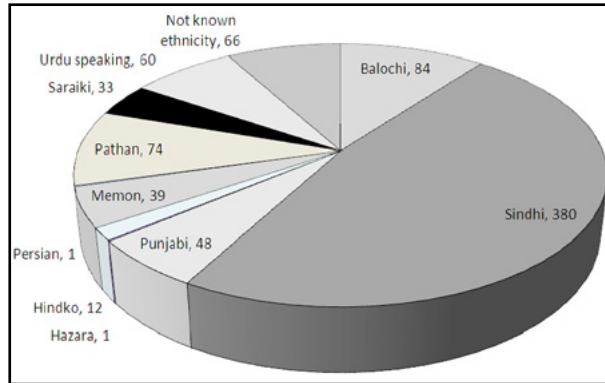


Fig.1: Frequency of beta thalassaemia in different ethnic groups in Pakistan.

RESULTS

The couples (Total number 798) included in the study came from different ethnic groups (Fig.1), 84 were Balochi, 380 Sindhi, 48 Punjabi, 1 Hazara, 12 Hindko, 39 Memon, 1 Persian, 74 Pathan, 33 Saraiki, 66 Indian immigrants, and 66 were of unknown ethnic groups. Prenatal diagnosis revealed that 224(28%) fetus had β -thalassaemia major. Four hundred (50.1%) had as β -thalassaemia minor. One hundred seventy three (21.6%) were healthy fetus and 1(0.12%) fetus had an undetected mutation. Most procedures (85%) were done between 12 and 14 weeks of pregnancy (range 10-16 weeks).

Most aspirations were easy; however in 20% of the cases the aspiration was difficult due to a variety of factors. Procedure related complications were seen in 20 cases (2.4%) and missed abortion occurred in six cases. Seven (3%) couples had refused to abort β -thalassaemia major fetus whereas 97% of fetuses were aborted as per recommendation. After the CVS procedure the detection of the β -thalassaemia mutation was done by ARMS PCR. The mutation was detected in 36% of Memon fetus, 34% of Balochi fetus, Saraiki 33.3% fetus, Punjabi 31% fetus, Pathan 28.4% fetus, Sindhi 28% fetus, Hindko

Table-I: Most Observed Frequency of Homozygous and Heterozygous gene Mutations in β -thalassaemia.

Homozygous mutation		Heterozygous mutation		
Mutation	N (%)	Mutation 1	Mutation 2	N (%)
IVSI-5	68(30)	Cd -15	Cap+1	7(3)
Fr 8-9	23(10)	Del 619	Cap+1	3(1.3)
Cd 15	7(3)	Fr 8-9	IVSI-5	5(2)
Cd 30	16(7)	Del 619	Fr 16	3(1.3)
Cd 5	2(1)	Del 619	IVSI-1	3(1.3)
Fr-16	7(3)	Del 619	IVSI-5	3(1.3)
Fr 41-42	7(3)	Fr-16	IVSI-5	3(1.3)

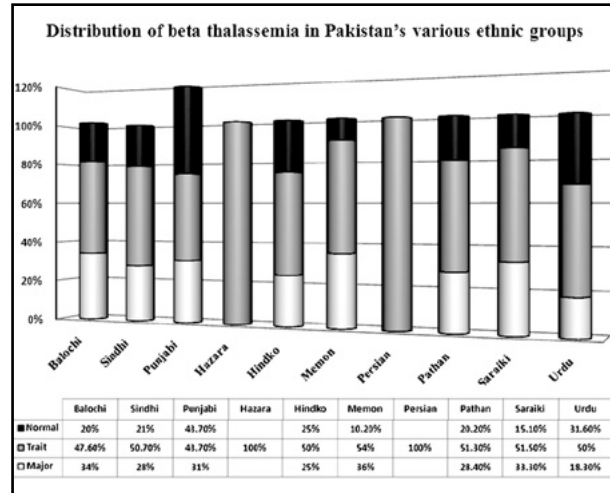


Fig.2: Distribution of beta thalassaemia in Pakistan's various ethnic groups.

25% fetus, and in Urdu speaking 18.3% foetus as major β -thalassaemia (Fig.2).

Consanguineous marriages are common in Pakistan and the pattern of consanguinity in different ethnic groups is shown in Fig.3. Among 732 couples, 552 (75%) were first degree, 55 (7.5%) were second degree and 125(17%) were third degree relatives. Homozygous mutations for major β -thalassaemia were found in 130 patients while 56 patients had heterozygous mutations. Table-I shows most common homozygous and heterozygous mutations of β -thalassaemia. Statistical Analyses was done using Statistical Package for Social Sciences (SPSS) version 17.

DISCUSSION

Thalassaemia is a heterogeneous group of genetic disorders of human haemoglobin synthesis, characterized by imbalanced globin chain

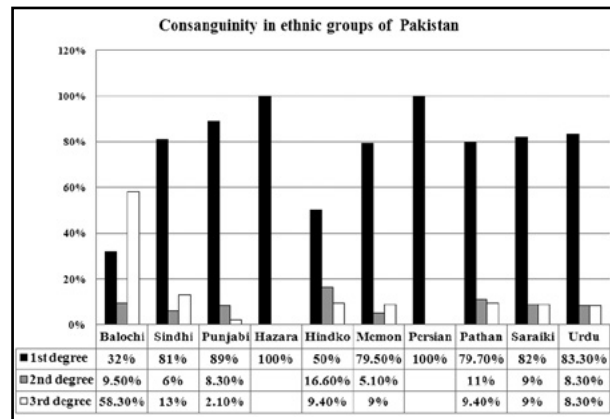


Fig.3: Consanguinity in CVS done of ethnic groups in Pakistan.

production which leads to ineffective erythropoiesis and anaemia.¹³ β -thalassaemia is highly prevalent in Middle East, India, Pakistan and South East Asia.^{14,15} The mean age of a thalassaemic child in Pakistan is 10 years. There are 25,000 children registered with thalassaemia federation of Pakistan however the actual figure is much higher which may be around one lac.¹⁶ Managing thalassaemia major children in Pakistan is creating a great burden on health care industry of the country. Multidisciplinary approach is needed to treat this disease but it is still becoming difficult for us due to shortage of resources and poor coordination among the existing facilities.¹⁷

Bone marrow transplantation, cannot be offered to most of the children in Pakistan just because they cannot afford it.^{18,19} Supportive therapy including regular blood transfusion and iron chelation therapy is the only treatment option left for children with thalassaemia in country like Pakistan.¹⁷ Awareness among the families having a child with β -thalassaemia will help to prevent the birth of more such children and will also help in providing comprehensive management to these children.²⁰ In resourceful countries like Sardinia²¹, the incidence of thalassaemia births was decreased from 1:250 to 1:1000 live births.

Similarly in Cyprus a reduction of 96% in incidence was observed. This was the result of proper attention given to the prevention program including carrier screening and pre-marital counseling.²² There have been some previous studies on the prevalence of β -thalassaemia in various ethnic groups of Pakistan. A study by Hafeez et al showed a high prevalence of disease in Punjabis followed by Sarikees and then Pathan.²³ In our study the disease was more prevalent in Sindhis as compared to other ethnic groups. The study conducted by Abdullah et al also showed a high prevalence of disease in Sindhis.²⁴ Cast wise we found the disease was most prevalent in Memon, similar results have been reported by Abdullah et al cast wise they also found high prevalence in Memon.²⁴

However, in Punjab, Hafeez et al showed that commonest cast with the disease was Rajput. The probable reason for this difference is that they conducted the study in Punjab while our study was conducted in Sindh i.e. the province with a higher population of Sindhis.²³ Immigrants from region of high carrier rates of thalassaemia and consanguineous marriages have increased the incidence of this recessive inherited disorder. In such communities, gene variants are trapped within extended families so that the affected

child is a marker of a group at high genetic risk.⁴ Each population at risk has its own (3-5) specific mutations. In this study, the two most common mutations were Fr 8-9 and IVSI-5. Similar results have been also reported.^{4,23}

In another study it was also observed that IVSI-5, Fr 8-9 and IVSI-I were the most common genetic mutation identified in Pakistan.²⁵ Cost of prenatal diagnosis can be curtailed by investigating the most frequently found genetic mutations in different ethnic groups in order to develop a short (population-specific) panel of primers of mutations. This will guide clinicians and families during genetic counselling and pregnancy termination decisions.²⁵ In our study 75% of the couples with the affected gene were first cousins. Consanguinity was quite high in the Punjabi population (89% couples were first cousin). This result is comparable (87.5%) with a study of Arif et al.²⁶

In another study it was also observed that 56% of the couples with the affected gene were first cousin. Hence, consanguinity increases the risk of disease and needs to be addressed in our region for better prevention of the disease.²³ In Pakistan CVS is a safe and effective tool for prenatal diagnosis and subsequent counselling in selected couples.⁶⁻⁸ Since initial reports of prenatal diagnosis in 1994, now this procedure is provided to at risk couples in three large cities including Karachi, Lahore and Islamabad.²⁷ Trans-abdominal CVS performed between 12-14 weeks of gestation was found to be safe technique. Procedure related complications were seen in 20 cases and missed abortion occurred in 6 out of 798 couples, 1% couples had refused to abort β -thalassaemia major affected fetus whereas 97% fetus was aborted as per recommendation.

We didn't find religious or cultural barriers as a limitation but lack of counselling in thalassaemia centers and no efforts on the government level are a major deterrent to the success full development of a thalassaemia prevention program. The babies detected to be heterozygous (carrier) or normal, were all delivered normally, and remained well throughout infancy, thus supporting the reliability of the diagnosis. Based on these results, it can be concluded that Ultrasound guided transabdominal CVS is an effective procedure for prenatal diagnosis of β -Thalassaemia in a Muslim community. We found no cultural hurdles for fetal sampling and prenatal diagnosis. This technique can play an important role in the prevention of thalassaemia. Inter-family marriages should be discouraged and if carriers do get married to each other, the prenatal

diagnosis should be done by CVS and termination of pregnancy should be advised.

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Author's Contributions:

SHA: Designed the Protocol, Performed CVS procedures and prepared the final manuscript.

TSS: Critically Reviewed the manuscript for final publication.

FNA: Contributed in manuscript writing.

KP: was involved in clinical management of patients,

GA: Sonologist was involved in clinical Assessment during procedures.

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