ORIGINAL ARTICLE

Prenatal Diagnosis of Thalassemia Major - an effective tool for thalassemia prevention in developing countries like Pakistan

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ABSTRACT

Background: Thalassemia is the most common, autosomal recessive, disorder of hemoglobin synthesis in the world. About 3 % of world population is carrier of beta thalassemia. Pakistan is located in Southeast Asia with 5.4% carrier rate and about 6000 children are born per year with thalassemia major.

Aims: To study the impact of providing easily accessible and free facility of prenatal diagnosis (PND) and termination of pregnancy (TOP) to at risk couples of having thalassemia major child.

Methods: The retrospective study was carried out to find the impact of providing accessible free facility of PND and TOP offered to 1869 at risk couples who belong to low socio economic group and rural areas of Punjab. After genetic counseling, trans-abdominal chorionic villous sampling under ultrasound guidance was carried out at 11-16 weeks of gestation. After aspiration of chorionic villi, DNA extraction was performed from parent's blood samples and chorionic villi.

Results: DNA analysis of 1869 CVS revealed 456 fetuses unaffected, 899 fetuses heterozygous and 514 beta thalassemia major. The miscarriage rate due to procedure of CVS was reported as 0.69%.

Conclusion: The study has found acceptability for free services of prenatal diagnosis in families at risk for beta thalassemia major children. Prenatal diagnosis has benefit of reassuring couple when results are normal.

Keywords: PND Prenatal Diagnosis, PTPP Punjab Thalassemia Prevention Program, CVS Chorionic Villous Sampling, TOP Termination of Pregnancy, PCR Polymerase Chain Reaction,

INTRODUCTION

Thalassemia is the most common, autosomal recessive, hemoglobin disorder in the world. About 3% of world population is carrier of beta thalassemia and approximately 60,000-70,000 children are born per year with thalassemia major[1,2, 3]. The reported carrier rate for Pakistan is 5.4% where about 6000 children are born per year with thalassemia major[4,5,6]. Large number of thalassemic children poses an immense burden on present health care system of Pakistan and also increases the physical and emotional sufferings among patients and their families. Worldwide, prevention of thalassemia is carried out by mass education, population screening, and prenatal diagnosis of at risk couples and termination of affected pregnancy [7]. Ahmad et al finds that thalassemia prevention in Pakistan can be carried out by extended family screening and offering prenatal diagnosis to at risk couples[8].

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Chorionic villous sampling is one of the invasive prenatal diagnostic methods[9]. This can be performed as early as 11 weeks of gestation through trans-abdominal or trans-cervical route under ultrasound guidance[10]. It has the advantage of giving relevant genetic information about fetus in first trimester of pregnancy and possible termination of affected fetus at an earlier stage.

Pakistan is an Islamic country and abortion is prohibited in Islam except if there is a serious risk to mother's health. A religious Fatwa was given by eminent Muslim scholars of Karachi, Pakistan in 1994[11] which states that if it is known that the fetus will be born with a serious genetic disease then the pregnancy can be terminated before 120 days of gestation[11,12]. The facility for prenatal diagnosis has been available in Pakistan since 1994[13], however its use remains limited due to lack of public awareness, cost involved, lack of availability of professional services and poor access[14].

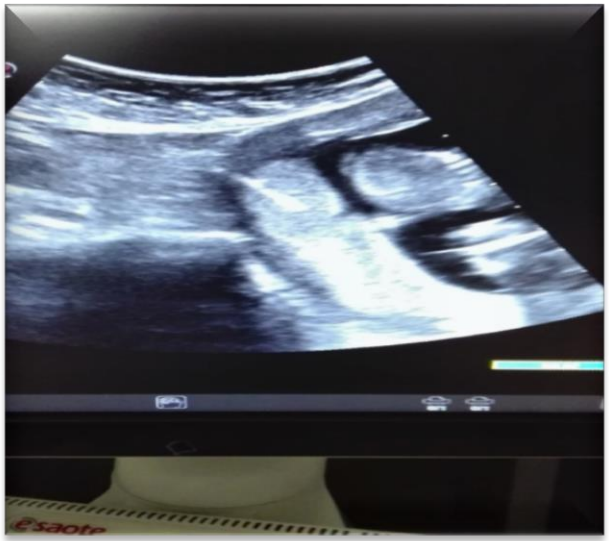
Considering this situation, the Government of Punjab initiated the Punjab Thalassemia Prevention Program in 2010 to provide free services of genetic counseling, carrier screening and prenatal diagnosis for the people of Punjab province with over 100 million populations. This study reports the experience of providing free services of PND and TOP offered to at risk couples through PTPP.

MATERIAL AND METHODS

This retrospective study was carried out to find the acceptability of prenatal diagnosis among at risk couples. Majority of these couples came from rural areas of Punjab belonging to poor socio economic groups and a low educational background.

The study was carried out by Punjab Thalassemia Prevention Program at its 4 regional centers, Sir Ganga Ram Hospital Lahore, Holy Family Hospital Rawalpindi, Nishter Hospital Multan and Bahawalpur Victoria Hospital Bahawalpur. 24 districts of Punjab are attached with these regional centers.

CVS needle in placenta



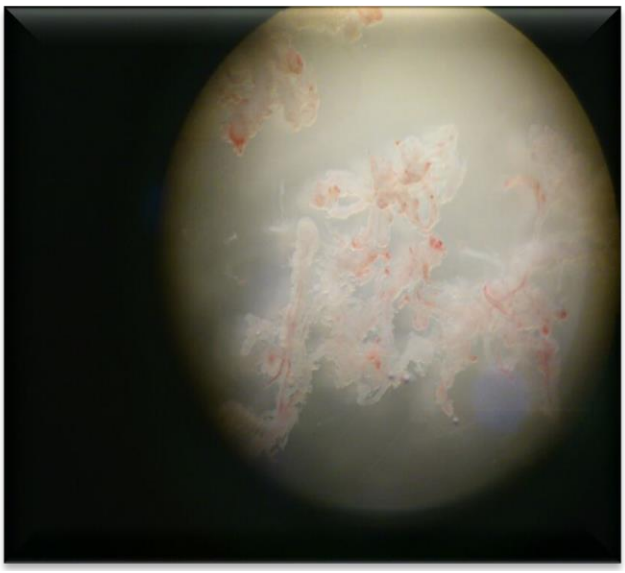
All the at risk couples were included in study who presented to PTPP centers from 2012 to March 2017. At risk couples were defined as those who had a thalassemia major child before. These couples were offered prenatal diagnosis at 11-16 weeks of gestation after genetic counseling. The couples having gestation more than 16 weeks were not included in study. Informed, written consent was taken from all couples.

A preliminary ultrasound was done to confirm singleton or twin pregnancy with viable fetus/ fetuses, placental localization, nuchal translucency and to rule out any obvious fetal anomaly which could be identified in first trimester of pregnancy.

All CVS procedures were carried out by trans-abdominal route under direct ultrasound guidance. A co-axial chorionic biopsy needle set 18G/20G (Rocket medical) was used. The 30ml disposable syringe was attached with 20 G needle and vacuum was created in syringe by pulling the plunger.

The to and fro movement of biopsy needle broke the chorionic villi and these were sucked in syringe. The sample was collected in normal saline in a patry dish and chorionic villi identified under dissecting microscope.

Chorionic villi



A post aspiration ultrasound was done for fetal viability and to rule out any retro placental hematoma formation or placental separation. Some patients experienced mild to moderate pain which was relieved by taking analgesics and rest. Patients were discharged 2 hours after the procedure and were advised to avoid long distance travelling for 24 hours. Prophylactic antibiotics were given for five days and Injection Anti D was given to Rh Negative mothers.

The chorionic villous samples were transported to DNA lab at Sir Ganga Ram Hospital Lahore on the same day from Lahore and on second day from other three regional centers in normal saline. The DNA was extracted from parental blood samples by GF-1 Blood DNA Extraction Kit (Vivantis) and from CVS (chorionic villi) by Chelax ® 100 Resin (Bio-Rad). Mutation analysis was carried out by PCR based allele specific ARMS. Termination of affected pregnancy was facilitated at all the four regional centers.

RESULTS

In this study, about 1869 prenatal diagnosis was performed from 2012- March2017. 1860 cases revealed common beta thalassemia mutations and 9 cases revealed rare mutations.

The maternal age ranged from 17-43 years. The mother's gestational age at the time of CVS procedure was 11-16 weeks. About 94% of couples belong to low socio economic group and 89.9% couples were illiterate. Consanguinity accounts for 86% and remaining 14% were non-consanguineous.

Table 1: Demographic variables of women undergoing CVS procedure (n=1869)

Characteristics	n
Age	
17-25	785(42%)
26-35	990(53%)
36-45	94(05%)
Parity	
0-4	1682(90%)
5-10	187(10%)
Gestational age (weeks)	
10	37(2%)
11	101(5.4%)
12	701(37.5%)
13	594(31.8%)
14	274(14.6%)
15 & above	162(08.7%)
Socioeconomic status	
<20,000	1757(94%)
>20,000	112(6%)
Education	
Nil	1862(89.9%)
Primary	98(5.3%)
Middle	54(3%)
Matric	18(0.96%)
F.A.	10(0.53%)
B.A.	5(0.21%)
M.A.	2(0.1%)
Consanguinity	
Consanguineous	1607(86%)
Non-Consanguineous	262(14%)

Table 2: Result of CVS performed for beta thalassemia (n=1869)

Beta thalassemia	n
Unaffected	456(24.4%)
Heterozygous	899(48.1%)
Homozygous	514(27.5%)
Termination of affected pregnancy	498(97%)
Refusal for termination	16(3%)
Miscarriage due to procedure	13(0.69%)

Table 3: Refusal for termination of affected fetuses

	n
Refusal of TOP of affected fetuses	16
Resistance from elderly of family	07
Pregnancy > 120 days	01
Refusal from husband	01
Fear of loss of 2nd twin	02

Unaffected fetuses were found to be 456; heterozygous 899 and 514 affected. Termination of 498 affected pregnancies was successfully carried out. The miscarriage rate due to procedure was 0.69% which is reported to be 0.2-2%, 0.86%, 0.98% and 1%[15,16 17].

Sixteen couples refused for termination out of which seven couples didn't agree to termination of pregnancy as the elder family members resist the decision. Six couple didn't opt for termination as pregnancy was more than 120 days and Islam does not allow terminating pregnancy beyond 120 days of gestation. One couple refused as husband was living abroad and not allowed his wife to terminate pregnancy. Two couples had twin pregnancy and result of mutation analysis showed one fetus heterozygous and other was homozygous. They refused selective fetal reduction due to fear of loss of second twin.

DISCUSSION

Prenatal diagnosis is found to be an effective tool in prevention of Thalassemia[11,8,19]. Chorionic villous sampling has the advantage of giving an early diagnosis in the first trimester of pregnancy. Few studies have been carried out to determine the acceptability of at risk couples for PND by CVS in our population [16, 20].The literature shows that prenatal diagnosis of Beta Thalassaemia by CVS is acceptable to Pakistanis as it reduces the burden caring an affected child on parents in a country where required resources are not available for the treatment and care of such affected children[5,20]. The PTPP is providing comprehensive free prevention services including awareness, carrier screening, genetic counseling and PND through CVS for thalassemia.

This study presents the data of PND through CVS of 1869couples at 4 regional centers. This is the largest experience of providing free facilities of PND at tertiary care hospitals near to their homes published from Pakistan. Previous studies from Pakistan have shown that the cost of the test and difficulty in access to PND were important reasons for the low uptake of PND in the country. The cost of PND is around 10,000 Rs (100 US$) in Pakistan[11], which is a very high cost in a country where the per capita income is 140,000 Rs (1400 US$) per annum. Since majority of at risk couples belong to low socio economic group (94%) and about 89.9% were illiterate, cost of the test and the time and money required to reach the prenatal diagnostic centers were found to be the major impediments[16]. PTPP has not only ensured that the facility of PND is provided totally free of cost but also near to their homes to all the at risk couples. It is now also available in all the four PTPP regional centers situated in North (Rawalpindi), Center (Lahore) and South (Multan & Bahawalpur) of the province.

Prenatal diagnosis is safe for the mother and fetus as it is performed in first trimester and under continuous ultrasound guidance. All the samples were collected in first attempt except few samples which required a second attempt of sample aspiration. This reduced the anxiety and pain associated with procedure. Fetal lose and other complications like retroplacental hematoma, placental

separation, ruptured amniotic membranes associated with chorionic villous sampling are minimal, making it acceptable for the at risk couples. The fetal loss rate in this study was 0.69% which is comparable to other studies where it is about 0.7%% and 1.5 %[21,17]. It is a safe and reliable technique showing the acceptability of free services of PND by the at risk couples.

The current data shows the main factors affecting the response of at risk couples are socio economic status, literacy, family pressures and consanguinity. These factors are also reflected by studies carried out in neighboring countries as India and Bangladesh[22,23]. The prenatal diagnosis was found to be technically feasible. It was accepted by all the families who have children with thalassemia major. Its acceptance is strengthened by the religious Fatwa taken by eminent scholars. It is difficult to differentiate between religious and social beliefs due to high rate of illiteracy. This problem is overcome by effective genetic counseling. The people were satisfied to know that Islam permits termination of pregnancy under special circumstances. Prenatal genetic testing has other benefits of reassuring couples at risk when results are normal and opportunity of pregnancy termination with an affected fetus. The termination of affected pregnancy was facilitated.

Health professionals play major role in providing information to thalassemia affected families. The misunderstandings about the cause of genetic disorders should be ruled out among people and correct information about the cause of genetic disease, resources available for its diagnosis, treatment and prevention should be provided. PTPP is providing ongoing genetic education and public awareness with free premarital and voluntary carrier screening in all parts of Punjab. Mass communication methods where and when required are delivered in regional and local languages.

CONCLUSION

Prenatal diagnosis of thalassemia is a valuable tool for its prevention, in any effective thalassemia control program. Free services of trans-abdominal, ultrasound guided CVS to at risk couples in first trimester of pregnancy are totally acceptable and help them to make informed decision for their family.

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