

Prevention programmes and prenatal diagnosis for beta thalassemia in Pakistan: A narrative review

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Abstract

Beta thalassemia in Pakistan is a serious health concern with an estimated 5-8% carrier frequency and birth of 5000 major children every year in the country. The treatment of beta thalassemia major patients poses a great economic burden; hence, the ideal approach towards this disease should encompass effective prevention services. At present only one government funded project "Punjab Thalassemia Prevention Programme" existed in Punjab province, and providing free of cost services for beta thalassemia screening and prenatal diagnosis. Complete blood count and haemoglobin electrophoresis remains the preliminary test for screening, while chorionic villi sampling and amplification refractory mutation system method have been most widely used for molecular diagnosis of beta thalassemia. Modern molecular techniques, non-invasive prenatal diagnosis, and pre-implantation diagnosis are in trial phases. In this review we have discussed the available diagnostic facilities and status of prevention programmes for beta thalassemia in Pakistan as well as future perspectives.

Keywords: Beta Thalassemia, Prenatal Diagnosis, Polymerase Chain reaction, Chorionic Villi.

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Introduction

Beta Thalassemia is the most prevalent autosomal recessive genetic disorder that results from errors in beta (β) globin gene and ultimately reduced synthesis of β -Globin chain. The worldwide carrier frequency of beta thalassemia is about 3% and it is estimated that every year averagely 60000 thalassemia major children are born all over the world.¹ In Pakistan beta thalassemia trait frequency ranges between 5-8%, thus there are more than 10 million carriers in country and every year, around 5000 children are diagnosed as beta thalassemia major in Pakistan.² Lack of awareness, illiteracy and consanguineous

marriages (70%) are the main factors leading to high carrier ratio in Pakistan.

According to World Health Organisation, if for any disease, the birth rate of affected infants exceeds 0.1/1000, an effective screening programme should be initiated.³

A recent Indian study has shown that the treatment cost of a thalassemia patient ranged from US\$ 629 to US\$ 2300 annually with an average cost of US\$ 1135 per patient,⁴ while in Pakistan, this would be higher and make thalassemia treatment a serious economic burden. Another aspect is that most of the patients belong to rural areas and have no access to the available health-care services as adequate blood transfusions with effective iron chelation. Bone marrow transplantation, the only cure for the disease, is affordable for just a few; hence, prevention rather than treatment has been demonstrated to be the successful planning to address this disease. Effectiveness of comprehensive national prevention programmes have been evidenced in a number of countries, for example Cyprus, Sardinia,⁵ several regions of Continental Italy⁶ and Greece.⁷ In a number of Muslim countries including Lebanon, Iran,⁸ Saudi Arabia, Tunisia, United Arab Emirates, Bahrain, Qatar, and Gaza Strip,⁹ a national policy for mandatory premarital screening aimed to limit carrier marriages. Unfortunately, in Pakistan, lack of a unified national policy for prevention of thalassemia have been undermined for many years, resulting an alarming increase in the number of beta thalassemia traits and birth of beta thalassemia major children. Currently a single government funded project, Punjab Thalassemia Prevention Programme (PTPP), in Punjab province is providing its services to people since 2009. At PTPP, available services include premarital screening, extended family screening, prenatal diagnosis and mutation analysis for beta thalassemia. A few numbers of private setups are also providing prenatal diagnosis on commercial basis with a cost of Pakistani Rupees (PKR) 8000 to 25000. Prenatal diagnosis for beta thalassemia has been underutilized in Pakistan for many reasons. Acceptance of prenatal screening has been hampered due to various factors like lack of awareness, illiteracy, social and religious beliefs.

Complete blood count (CBC) and haemoglobin (Hb)-electrophoresis remain the basic screening tests for beta

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thalassemia. For prenatal test, chorionic villi sample (CVS) is taken by trained gynaecologist for molecular diagnosis and mutation analysis is done mostly through amplification mutation refractory system (ARMS) or allele specific-polymerase chain reaction (PCR) method. ARMS-PCR method is a very effective technique, and widely used method for mutation detection of beta thalassemia. However DNA sequencing, a gold standard methodology, has been used to screen unknown mutations. New molecular diagnostic methods like high resolution melt curve (HRM) analysis, fluorescent labeled probes and pre-implantation studies are being used on trial basis and will be available in near future. In this article we have summarized the status of available prevention programmes and prenatal diagnostic facility for beta thalassemia in Pakistan as well as future perspectives.

Prevention versus Treatment

Economic cost for treatment of thalassemia is immense.¹⁰ A recent Indian study has mentioned the treatment costs of a thalassemia patient at an average of US Dollars 1135 yearly,⁴ whilst in Pakistan, the estimated cost of treatment for beta thalassemia major (BTM) would be higher. Previously it has been estimated that in Pakistan, the average cost for comprehensive treatment of 60,000 registered patients is approximately 7.8 billion rupees per year. The cost of blood transfusion is Rs. 30,000, whereas for iron chelation therapy is Rs. 150,000 per year per child.¹¹ As majority of affected patients belong to poor families and cannot afford this costly treatment, a number of non-government organizations (NGOs) are making efforts to provide blood transfusion along with iron chelation therapy to affected children free of cost. Bone marrow transplantation is considered the only way to cure this

disease, but due to very high cost it remained beyond the reach of poor affected families. In this situation, prevention is most feasible option to effectively deal with this disease.

In Table-1, comparison between the cost of treatment and prevention clearly show the difference in terms of economic burden. However, this is merely a rough estimate, it still gives an obvious understanding that cost of prevention seems far less than treatment, which makes prevention a better strategy to chose for reducing the overwhelming economic burden among beta thalassemia affected patient's treatment in future.

Available diagnostic services for beta thalassemia in Pakistan

Screening Tests for beta thalassemia

CBC with peripheral smear and Hb electrophoresis are considered baseline tests for beta thalassemia screening. In BTM, there is a marked reduction of haemoglobin concentration, microcytic hypochromic anaemia, with nucleated red blood cells on peripheral smear and Hb-electrophoresis indicates reduction in haemoglobin A with variable increase in foetal haemoglobin (HbF). On the other hand, individuals with beta thalassemia trait are usually asymptomatic and diagnosed incidentally by presence of microcytic hypochromic red blood cells and mild anaemia. A person is considered as beta thalassemia carrier if there are decreased values of RBC indices (MCV, MCH, MCHC) than normal, red blood cells >5.0 millions/cm on CBC and haemoglobin A₂ >3.5 on Hb electrophoresis, while excluding iron deficiency anaemia with evaluation of ferritin level.¹²⁻¹⁸

Table-1: Comparison of Cost of treatment and Cost of prevention for beta Thalassemia.

Cost of Treatment		Cost of prevention	
Short term economic burden			
Number of new beta thal major cases per year	5000	Total population of Pakistan	>200 millions
Cost of treatment per patient	~ 236,532	Birth rate	~19/1000 population= 2.9%
Cost of treatment for 5000 patients per year	~ 1182660 millions	Total no. BT carrier	~ 12 million (5%-8% carrier rate)
Total number of BTM patients	~60000	No. of pregnancies in carrier couples per year	2.9% x 12 = .36 million (birth rate% x carrier population)
Cost of treatment of 60000 patients per year	~14191920 Millions	No. of prenatal test required per year	Total no of pregnancies in carrier couples per year =360000 36000/12=30000
Long term economic burden		Number of Prenatal test required per-month	~5000 PKR
Average life expectancy in Pakistan	~ 10 years	Cost of prenatal test per patient	5000 x 30000=150 million PKR
Cost of treating 5000 patients for 10 years	~ 11826600 millions	Cost of prenatal tests per year	150 x 10 =1500 million PKR
Cost of treatment of 6000 BTM for 10y 1182660 million PKR		For a 10year prevention program	Cost of Prevention for a 10y program 1500 million PKR

CVS= Chorionic villi sample, ARMS= Amplification refractory mutation system, PTPP= Punjab Thalassemia Prevention Program, HRM= High Resolution Melt Curve Analysis, Hb= Hemoglobin, CBC= Complete blood count, BTM= Beta Thalassemia major, BTT= Beta Thalassemia trait, Cff-DNA= Cell-free fetal, VNTR= Variable number tandem repeats, STR= Short Tandem repeats, MCC= maternal cell contamination.

However, in some cases with borderline values, a confirmed diagnosis becomes difficult and in these instances, a genetic molecular analysis to screen any silent mutation (e.g. CAP+1) is recommended for a definitive diagnosis.¹⁹⁻²²

Nevertheless, while making a definitive diagnosis, patient's clinical history, family history of beta thalassemia, any underlying condition like iron deficiency anaemia or pregnancy must be considered.

With increasing awareness about beta thalassemia in the country, it is now an emerging trend among gynaecologists and paediatricians to recommend suspected couples to be analyzed for beta thalassemia carrier screening. CBC is relatively a cost-effective and widely available test in almost all urban and rural government health set-ups; however, Hb-electrophoresis is mostly available at district and tertiary care hospitals.

Prenatal diagnosis and genetic counseling for beta thalassemia

Since many years, prenatal diagnosis has been used to offer an accurate and prompt molecular characterization of the couple at risk and intended to detect foetal abnormalities during early gestational age of foetus. Genetic counseling is provided to both members of the couple at risk in an unobtrusive way. The couple is informed in detail about nature of the disease, implications of being carriers, nature of test and possible risks.²³ Most importantly, they are convinced for termination of pregnancy in case of affected foetus.

Foetal DNA analysis is done by obtaining sample from either amniotic fluid or chorionic villi. Presently, the most widely used and acceptable practice is examination of chorionic villi specimen (CVS), as the method have an advantage of being carried out during first trimester of pregnancy and comparatively more safe than amniocentesis. An expert gynaecologist takes chorionic villi sample with ultrasound guided specialized needle generally at 10th-12th week of gestation.²⁴⁻²⁶ However, the risk of foetal mortality associated with both methods has been reported as 1-2% and this possible risk is also explained to the couple before CVS test.

Foetal DNA analysis and ARMS PCR

Beta-thalassemia is caused mostly by point mutations or, more rarely, deletions in the beta globin gene. Molecular characterization of Foetal DNA have been performed by different methods like, PCR Restriction Fragment Length Polymorphism, allele specific or ARMS PCR, high resolution melt curve analysis (HRM) through real time PCR and direct DNA sequencing of beta globin gene. Among these methods, ARMS-PCR has been used most widely for

prenatal testing of beta thalassemia in developing countries. ARMS-PCR is a powerful, sensitive and very cost effective method to diagnose single base pair change in DNA. In ARMS-PCR, two primers are designed in such a way that there will be mismatch at the 3-end of primer sequence, which hinder its annealing to template. The mutation primer can only anneal to the DNA sequence with mutation and not with normal DNA sequence, while in case of normal primer it will be vice versa.

In this method, the target DNA fragment is amplified in two separate PCR reactions using a common primer and either of the two following primers: mutation specific primer that is complementary to the mutation to be identified and normal primer that complementary, at the same location, to the normal DNA sequence. DNA from a normal person will be amplified only by the normal primer, while DNA from beta thalassemia homozygotes only by the mutation specific primer and DNA from heterozygotes will be amplified by both primers. The co-amplification of a different sized fragment of β -globin gene is simultaneously done as an internal control of PCR reaction.²⁷ This is followed by agarose or polyacrylamide gel electrophoresis to analyze the results.

As described earlier that CVS testing is an invasive procedure, therefore, foetal samples obtained by amniocentesis or chorionic villus sampling (CVS) are at risk of contamination by maternal DNA due to the presence of maternal blood or maternal tissue. The potential presence of maternal cells in CVS samples may lead to a significant pre-analytical risk and is most common cause of error in a haemoglobinopathy foetal prenatal diagnosis. When a CVS sample is received for prenatal diagnosis, in the laboratory, it is essential that any maternal tissue present in the foetal sample is carefully removed. Therefore, it is highly recommended to determine pure foetal origin of all prenatal specimens, undergoing genetic analysis and maternal cell contamination (MCC) analysis should be performed in parallel with diagnostic testing. MCC testing is required in each case, or at least when foetal diagnosis result is identical with maternal result, to identify any maternal DNA contamination present.²⁸

The most widely used method is based on the use of highly polymorphic short tandem repeats (STR) polymorphism to discriminate between foetal DNA from maternal DNA (that may have contaminated CVS sample). Although this is very sensitive technique, but commercially offered kits are very expensive and their use in routine prenatal testing is unlikely in developing countries, because of the increase in test cost. An alternative cost effective approach, though not sensitive as STR, is the use of variable number tandem repeat (VNTR) polymorphism²⁹ to check maternal cell contamination in CVS sample.

In Pakistan, ARMS-PCR is being utilized for prenatal testing for last 20 years. Although the method is quite effective, but it can only be used to detect known mutations that is a major limitation to use ARMS-PCR solely in prenatal testing. Additionally, prenatal testing is a time constraint test and decision to terminate or continue the pregnancy depends upon the timely confirmation of foetal diagnosis.

Current prevention measures for beta thalassemia in Pakistan

Currently in Pakistan, not a single prevention programme and unified policy at national level is available to counter this disease. However, at provincial level some initiatives have been taken, legislation has been approved for premarital screening in Sindh, KPK and Baluchistan, but implementation remains the issue. In Sindh, a very few number of laboratories are offering prenatal testing, but it may cost around Rs.20,000 to 30,000, which is beyond the limit of the common man. To date, there is no existent prevention programme for beta thalassemia in KPK and Baluchistan, despite the fact of high carrier frequency in these provinces because of tribal culture for consanguineous marriages.

Punjab is the largest province of Pakistan with almost 110 million population. In Punjab, the first prenatal beta thalassemia test was conducted in 1994 in Armed Forces Institute of Pathology (AFIP), Rawalpindi.³⁰ In capital city Islamabad and Rawalpindi few setups are providing this facility in the range of 8000 to 12000 Pak Rupees per patient. National Institute of Genetic Engineering also started prenatal testing³¹ for a short period as a research project and later on discontinued this facility due to shortage of funds. However, all these organizations are working on a very small scale, not enough to cover 110 million population of Punjab.

Punjab Thalassemia Prevention Programme

Punjab is the only province that has taken a lead to initiate a public sector prevention programme in 2009 as 'Punjab Thalassemia Prevention Programme (PTPP)'.

PTPP is providing its services in 36 districts of Punjab through its three regional centers, Holy Family Hospital Rawalpindi, Victoria Hospital Bahawalpur and Nishtar Medical College Multan, linked to a central operational head office at Sir Ganga Ram Hospital Lahore.³²⁻³³

This project is providing services of premarital screening, general population screening, extended family screening, and most importantly prenatal diagnosis of beta thalassemia. The beauty of this programme is that it is providing all these services free of cost and hence proving a blessing to the poor families affected with beta thalassemia.

At district level, CBC and haemoglobin variant studies by high performance liquid chromatography (HPLC) or Hb electrophoresis have been provided as screening tests, while prenatal diagnostic facility is available at three regional centers and head office. Field offices have been appointed in all districts to facilitate the outreach families to be screened for beta thalassemia.

Prenatal diagnostic facility is available to couples at risk. A detailed genetic counseling is provided to the carrier couples and they are informed comprehensively about nature of the test, implications of being a carrier and convinced for termination of pregnancy in case of affected child.

PTPP has a panel of experts and trained gynaecologists for CVS sampling. Foetal CVS sample is taken by ultrasound guided specialized needle during the 10th-12th week of pregnancy and sent to head office. A careful dissection of foetal tissue is carried out to remove any maternal decidua under microscope. A well-equipped DNA lab is present at head office of PTPP that perform molecular analysis of foetal DNA sample and results are available within 15 days of sample receiving, well before the 17th week of pregnancy.

An in-house developed assay, based on VNTR analysis, is part of the standard operating procedure to check every CVS sample that reported as trait, to minimize the chances of misdiagnosis because of maternal contamination.

While the report has been issued a follow up process is also carried out to ensure termination of pregnancy in case of affected foetus. Physicians from different hospitals also send suspected cases for genetic analysis and confirmatory diagnosis of beta thalassemia and other beta globin variants such as Hb-D Punjab, HbS as well as Xmn-I polymorphism, BCL-1 polymorphism.

This programme has also made great efforts through screening camps at different institutes, symposiums, seminars and walks to raise awareness about thalassemia among general public.

Although PTPP is doing a great job, this programme also have some problems in terms of unavailability of funds, limited resources and have not a dedicated space to carry out its operations for all above mentioned services.

Future Perspectives for the Prevention of Beta Thalassemia

In Pakistan, beta thalassemia trait frequency ranges between 5-8%, hence, more than 9.8 million carriers in country and around 5250 children are diagnosed with beta thalassemia each year in the country. The situation may be worse in the absence of an effective national

policy for prevention of beta thalassemia, in future. A single prevention programme with limited resources only in one province is inadequate for large scale preventive measures. Treatment of beta thalassemia major patients poses an immense economic burden and enormously necessitates the need for disease prevention programmes like PTPP, to be initiated in each province.

Although CV sampling is an easily adaptable and efficient technique, however, the invasiveness may lead to a risk of miscarriage. Therefore, adaptation and development of innovative, safe and highly valuable prenatal diagnostic methods should be considered in future. Noninvasive prenatal diagnosis such as Cell-free foetal DNA (CFF-DNA) in maternal plasma and foetal cell in maternal blood are new emerging trends and being used successfully in many countries. Pre-implantation genetic diagnosis is an alternate to prenatal diagnosis in which couples at risk have to undergo for in vitro fertilization and then embryos are evaluated to determine for any genetic abnormality after that selected disease free embryos placed in uterus to carry on pregnancy. Although these are less risk prone procedures as compared to invasive CVS, need very expensive instruments, sophisticated techniques and much more experience.

Modern molecular techniques for efficient and cost effective prenatal diagnosis should be adopted in future. HRM a very sensitive technique, which is able to detect even a single base pair change in DNA sequence has now been in clinical use for genetic analysis of beta thalassemia in a number of developing countries like India, Bangladesh and Thailand. Another effective technique is using fluorescent labelled probes to detect the known beta thalassemia mutations by Real Time PCR. Both of these methods need no post PCR analysis by gel electrophoresis and hence are time saving techniques. From PTPP resources, it has been announced that HRM and fluorescently labeled probes Real time PCR are in trail phase and will be available soon in near future for clinical purpose. However, the limitation of these methods is to detect only known mutations. DNA sequencing remain the Gold standard technique to detect the unknown changes in DNA sequence and have been widely used as routine diagnostic method for beta thalassemia in most of the developed countries. Despite the fact these techniques are very sensitive, efficient and time saving as compared to ARMS method, their use as routine diagnostic method is hindered due to high cost equipment and reagents required for these methods. Government should provide funds to current prevention setups for using these advanced methods in future that will aid in effective prevention plan for this disease.

Conclusion

A comprehensive long term prevention plan is the utmost necessity to extirpate or at least minimize the likelihood devastating spread of this disease in future.

Below is a proposed plan of action for effective prevention planning of beta thalassemia.

I. A uniform national policy for mandatory premarital screening.

II. Initiation and establishment of 10-15 year prevention programmes in each province operating under a national prevention programmes for beta thalassemia.

III. Population based screening and development of thalassemia registry to get authentic thalassemia carrier frequency that will help in future prevention planning in a more productive way.

IV. Pre-natal diagnosis and appropriate genetic counselling should be available to every couple at risk and in case of affected foetus; termination of pregnancy should be part of the prevention programme.

V. A consensus religious policy regarding prenatal diagnosis and termination of pregnancy should be established.

VI. Countrywide mass awareness should be provided about the disease by means of national media, social media, conferences, seminars, walks in urban areas, while in rural areas through camps and special counters in district and tehsil hospitals.

VII. Establishment of prevention centers along with treatment centers that will make an easy access for the effected families to both platforms.

VIII. For a successful prevention programme, support from all stakeholders including religious scholars, electronic and print media, gynaecologists, paediatricians, thalassemic families, government and non-government organizations is required.

We hope that by working on multiple grounds simultaneously and with devotion, will ultimately decrease the incidence and prevalence of beta thalassemia in Pakistan.

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