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PROFICIENCY OF PRENATAL MOLECULAR DIAGNOSIS FOR B-THALASSEMIA AMONG CARRIER COUPLES

Adil Rahim[1], Sultan Mehmood Khan[2], Sohail Akhtar[3], Fawad Ali[4], Shoaib Ali[5*]

[1]*Clinical Technologist Pathology at Khyber Medical College Peshawar, Khyber Pakhtunkhwa (KPK), Pakistan.*
[2]*Medical laboratory Scientist at Alkhidmat Diagnostic Center Miranshah. Pakistan.*
[3]*M.Phil Medical Lab Science (MLS) Scholar at Abasyn University Peshawar, Khyber Pakhtunkhwa (KPK) Pakistan.*
[4]*BS Medical Laboratory Technology (MLT) Student at Jibran College of Health Sciences and Institute of Nursing Swat, Khyber Pakhtunkhwa (KPK) Pakistan.*
[5*]*Lecturer MLT at Iqra, National University (Swat Campus) Khyber Pakhtunkhwa (KPK) Pakistan*

[1]adilrahim9066@gmail.com, [2]sultanmehmoodinc@gmail.com, [3]sohailakt873@gmail.com, [4]faadikhan148@gmail.com, [5*]ashoaib0313@gmail.com

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Shoaib Ali

Abstract

Background: *Consanguineous marriage and inadequate genetic services are prevalent in Pakistan and one of the most prevalent genetic disorders of hemoglobin is β-Thalassemia which is a major public health concern in the Khyber Pakhtunkhwa (KP) area of Pakistan.*

Objectives: *To assess the attitude, awareness and practice of prenatal diagnosis in beta thalassemia carrier couples of Chorionic Villus Sampling (CVS.*

Materials and Methods: *The study was cross sectional study which included 600 married couples with β-thalassemia carrier status from Alkhidmat Thalassemia Centers (ATCs). Structured interview administered questionnaire on socio-demographic characteristics, disease history, knowledge, attitude, practices and barriers towards prenatal molecular diagnosis were used to collect the data.*

Results: *This study proves that there is still a lack of awareness about prenatal molecular testing and CVS. The main barriers was lack of awareness, financial limitation, limited access to specialist diagnostic facilities, fear of invasive techniques, cultural beliefs, religious concerns and lack of genetic counseling. The higher educational level, higher knowledge scores, being urban residents and use of genetic counseling were significantly correlated with use of prenatal molecular diagnosis.*

Conclusion *There was significant difference between awareness and utilization of prenatal molecular diagnosis among β-thalassemia carrier couples in KPK. Therefore, the public awareness towards the disease, strengthening the genetic counseling service, increasing the availability of prenatal molecular test and making the screening service accessible to all programs in public health are crucial to reduce the occurrence of β-thalassemia major in the province.*

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INTRODUCTION

Beta thalassemia, the second most common cause of hemoglobinopathy is a serious public health problem in developing countries. This is due to mutations in the beta-globin gene (HBB) which lead to decreased production or even the complete lack of the β-globin chain (1). Globally 1.5% of population are traits of beta thalassemia and over sixty thousand childes get diagnosed with this sever hemoglobinopathy with highest prevalence rate in Southeast Asia (2). Pakistan has a huge burden of β-thalassemia. The prevalence of β-thalassemia mutations in Pakistan is estimated to be 5–7% and the total number of β-thalassemia carriers in the country is almost 9–12 million (3,4). Pakistan is one of the countries with high disease burden with 5000-9000 children born with β-thalassemia major per year (5). The number of people affected strains health care institutions, the blood transfusion services, and affected families. The situation of β-thalassemia is worse in Khyber Pakhtunkhwa (KP) as large families, inadequate awareness of the disease and lack of genetic counseling services play significant roles in perpetuating the disease. Carrier screening and prenatal diagnostic services are not known to many families living in rural and underserved communities. That is, children are being born with these disabilities when there are prevention techniques available (6). One of the many causes of high prevalence of β-thalassemia in Pakistan is consanguineous marriage. Consanguinity rates have been reported to be as high as 50–70% in Pakistan, which plays a significant role in the transmission of β-thalassemia and other inherited disorders (7).

Prenatal molecular diagnosis is one of the best management tools used to prevent children from being born with the diagnosis of β-thalassemia major. A method to identify the carriers for a condition in early pregnancy and allow informed reproductive decisions (8). In the case of β-thalassemia chorionic villus sampling (CVS) is thought to be the most widely used type of prenatal testing. It is normally performed between the 10th and 13th week of pregnancy, and there are no x-rays used in it. Ultrasound guidance is used during the procedure, which takes place at the 10th to 13th weeks of gestation. Some amount of placental tissue carrying the DNA of the baby is taken through either the trans-abdominal or trans-cervical technique. In the course of the molecular analysis of the chorionic villi, there is enough fetal DNA to extract the baby's DNA (9). The diagnosis of this disease is basically the testing of mutation in HBB gene on chromosome 11 (10). The early diagnosis provides an opportunity to provide adequate genetic counseling and informed decision-making and reduces the emotional and medical complications of late stage pregnancy management (11). With the use of prenatal molecular diagnosis programs, the prevalence of β-thalassemia major has been drastically decreased in some countries. Comprehensive carrier screening, genetic counselling and prenatal diagnostic services have proven to be highly effective in preventing affected births in countries like Cyprus, Greece, Italy and Iran (12). Although there are molecular diagnostic tests available for the prediction of β-thalassemia in utero, not all carrier couples are aware of and utilize these services in Pakistan. There are various socio-economic, cultural, religious, educational and healthcare-related obstacles that could hinder couples from seeking prenatal diagnosis during pregnancy.

There are several studies that have assessed the prevalence of β-thalassemia and awareness of the public in the country about the necessity of carrier screening in Pakistan, but little is known about knowledge, attitudes and utilization of prenatal molecular diagnosis in β-thalassemia carrier couples in Khyber Pakhtunkhwa province. However, most existing studies are related to the prevalence of the condition, carrier frequency and management of blood transfusion, with little research on awareness and acceptance of CVS-based prenatal diagnosis. Thus, it is important to study prenatal molecular diagnostics knowledge and factors affecting its use among the carrier couples in this area.

2. MATERIALS AND METHODS

This study was a cross-sectional descriptive study of the couples of β-thalassemia carrier in Khyber

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Pakhtunkhwa Province in Pakistan. This study was conducted in Alkhidmat Thalassemia Centers and various hospitals of the respective districts in Khyber Pakhtunkhwa Province from January 2025 to June 2026. The study population consisted of married couples in which the husband and wife were each tested for β-thalassemia and both confirmed to be carriers. Only those who wanted to participate were included, the remaining who were not careers, had no record or refused consent was not included. The number of samples was calculated based on the WHO formula for single population proportion. With 95% confidence level, 5% margin of error and 50% awareness, the minimum sample size of 384 was selected. The sample size was raised, however, to 600 participants to increase the statistical power and representativeness. The selected centers were used to recruit the eligible participants by using consecutive sampling technique until the required sample size was reached. The data were collected by means of a structured and interviewer-administered questionnaire which contained information on socio-demographic data, knowledge of prenatal diagnosis, attitude towards prenatal testing, use of prenatal molecular diagnosis using the chorionic villus sample and the barriers to the use of prenatal molecular diagnosis. All participants gave written informed consent and the study was approved by the institutional review committee. The data was analyzed by SPSS version 26. Data were represented in frequencies and percentages, and the correlation between variables was tested by Chi-square test and logistic regression analysis. P value < 0.05 was considered statistically significant. Tool Structured interview-based questionnaire, developed after reviewing literature, was utilized to collect data.

Variables Utilization of prenatal molecular diagnosis (Yes/No) was the dependent variable. Age, education, type of residence, income, family history of thalassemia, No. of affected children, Knowledge score and seeking of genetic counseling were independent variables. Descriptive statistics (Frequency, percentage, mean and SD) have been applied to summarize the data. The Chi square test was used to evaluate the association among variables and logistic regression was used to determine the predictor of utilization. A p-value < 0.05 was considered statistically significant.

3. RESULTS

In this study 600 β-thalassemia carrier couples were enrolled in the study from Alkhidmat Thalassemia Centers and affiliated hospitals of Khyber Pakhtunkhwa. Most of the participants were young (25-35 years), and were from rural areas as shown in. The majority of respondents were poorly or moderately educated and there were a significant number of respondents who were illiterate. Most of the participants had low income jobs and the majority of families were in the low socioeconomic status. The study population exhibited high level of consanguineous marriages which is common in the region. There was a low level of overall knowledge of prenatal molecular diagnosis. Many of the respondents did not know of prenatal diagnosis or Chorionic Villus Sampling (CVS). Limited was also the awareness of the fetal presence of β-thalassemia in the fetus and its prenatal testing, which occurs early in pregnancy. The overall distribution of participants was mostly in the poor to moderate range. The attitude towards prenatal molecular diagnosis was generally favorable, although the level of knowledge was low. The majority of respondents indicated that prenatal testing is good for carrier couples and that genetic counseling should be integral to the provision of reproductive health care services. Most of the participants had not had genetic counseling before getting pregnant [Table 1].

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Table 1: Demographic, Disease related information, Knowledge of Prenatal Diagnosis, Attitude Toward Prenatal Diagnosis, Practice and Utilization of the participants of the study

Variable	Category	Frequency	Percentage %
Age (years)	<25	120	20.0
	25–35	310	51.7
	>35	170	28.3
Residence	Rural	420	70.0
	Urban	180	30.0
Education	Illiterate	180	30.0
	Primary	210	35.0
	Secondary	150	25.0
	Higher	60	10.0
Monthly Income	Low	380	63.3
	Middle	170	28.3
	High	50	8.4
Family History of Thalassemia	Yes	410	68.3
	No	190	31.7
Affected Children	None	210	35.0
	One	260	43.3
	Two or more	130	21.7
Consanguineous Marriage	Yes	480	80.0
	No	120	20.0
Heard about Prenatal Diagnosis	Yes	240	40.0
	No	360	60.0
Know about CVS	Yes	180	30.0
	No	420	70.0
Know Prenatal Test Detects Fetal Thalassemia	Yes	200	33.3
	No	400	66.7
Know Test is Performed During Pregnancy	Yes	220	36.7
	No	380	63.3
Know Where Test is Available	Yes	190	31.7
	No	410	68.3
Knowledge Level	Poor	340	56.7
	Moderate	210	35.0
	Good	50	8.3
Prenatal Diagnosis is Beneficial	Agree	480	80.0
	Neutral	80	13.3
	Disagree	40	6.7
Carrier Couples Should Undergo Testing	Agree	450	75.0
	Neutral	90	15.0
	Disagree	60	10.0
Genetic Counseling Should be Mandatory	Agree	500	83.3
	Neutral	60	10.0
	Disagree	40	6.7
Utilized Prenatal Diagnosis	Yes	150	25.0
	No	450	75.0

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Received Genetic Counseling	Yes	170	28.3
	No	430	71.7

Several barriers were found that explain why prenatal molecular diagnosis is not used. The most frequently mentioned barriers were financial, lack of knowledge, limited access to specialized diagnostic facilities, fear of invasive procedures (CVS), cultural/family pressure and poor physician recommendation. In a few instances religious issues were also factors. Healthcare providers, especially doctors from the thalassemia centers were the main source of information on prenatal diagnosis. Family/friend (20%), media and social (10%) were other sources. Formal genetic counseling services were found to be underutilized. Factors associated with non-performing prenatal molecular diagnosis were lack of knowledge, financial constraints, location and high costs of service, fear of CVS procedure, cultural barriers and provider recommendations [Table 2].

Table 2: Barriers to Utilization, Source of Information, Reason for Not Undergoing Testing of the Participants

Variable	Category	Frequency	Percentage %
Barriers to Utilization	Lack of Awareness	380	63.3
	Financial Constraints	300	50.0
	Lack of Access	260	43.3
	Family Pressure	240	40.0
	Fear of CVS Procedure	220	36.7
	No Physician Recommendation	200	33.3
	Religious Concerns	180	30.0
Source of Information	Doctor	260	43.3
	Thalassemia Center	180	30.0
	Family/Friends	120	20.0
	Social Media	90	15.0
	TV/Radio	60	10.0
Reason for Not Undergoing Testing	Lack of Awareness	320	71.1
	Financial Constraints	260	57.7
	Lack of Access	240	53.3
	Fear of CVS Procedure	200	44.4
	No Physician Recommendation	190	42.2
	Cultural Restrictions	180	40.0
	Religious Concerns	160	35.6

There were significant associations between utilization of prenatal molecular diagnosis and higher education level, urban residence, higher income, better knowledge scores and genetic counseling [Table 3].

Table 4: Logistic Regression

Variable	OR	95% CI
Education	2.4	1.6–3.8
Urban residence	1.9	1.2–3.1
Knowledge score	3.2	2.1–4.8
Counseling	4.5	2.9–6.8

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The division wise distribution of the couples are listed in the Figure 1.

Division	Couples
Peshawar Division	110
Mardan Division	90
Malakand Division	83
Hazara Division	55
Kohat Division	83
Bannu Division	55
Dera Ismail Khan Division	83
FATA	41
Total	600



Figure 1 Division-wise Participant Distribution from Khyber Pakhtunkhwa

4. Discussion

The current study was conducted to evaluate the awareness, attitude and utilization of prenatal molecular diagnosis among β-thalassemia carrier couples in Khyber Pakhtunkhwa, Pakistan. The results showed that the general awareness on prenatal diagnosis and Chorionic Villus Sampling (CVS) was low, and the use of pre-natal testing was still relatively low even though there was a positive attitude in most cases.

The low awareness noted in the present study is consistent with previous studies in Pakistan where lack of awareness about the prenatal diagnosis among carrier couples has been reported. Low uptake and poor awareness of genetic counselling services among high risk families in Pakistan was reported by Ahmed et al indicating that there is poor health education and no structured screening programs (4). The same is true in other parts of Pakistan, in which studies report that although the prevalence of β-thalassemia is high, utilization of prenatal diagnosis is very low, owing to unawareness and accessibility problems (13).

Iran, on the other hand, has shown much more prenatal diagnosis utilization, owing to its well-established national thalassemia prevention program. Research from Iran shows that the use of structured carrier testing, pre-marital counseling and free prenatal diagnosis has resulted in a significant decrease in the number of children born with β-thalassemia major (12). Iran illustrates the need for national level screening and awareness programs.

In Saudi Arabia, there are reports of moderate knowledge of genetic disorders and low uptake of prenatal diagnosis, presumably because of cultural and religious beliefs. Prenatal diagnosis is required, although the compliance to the screening programs is affected by individual and family choices and there is some resistance to pregnancy termination even if the information reveals severe fetal disease (14).

Similarly, in India, limited awareness exists among carrier groups, especially in rural areas. Few genetic counseling services are available and financial issues are identified as barriers. However, national thalassemia control programs and involvement of NGOs are gradually improving people's awareness (15).

The better results has been observed in Turkey compared to Pakistan, with the presence of an integrated national screening program and availability of genetic counseling. Research

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suggests there is a greater level of knowledge and better use of prenatal diagnosis with regional differences (16).

The increased proportion from Peshawar and Mardan Divisions may be attributed to the presence of major tertiary care hospitals and specialized thalassemia centers, which are a referral source from all over KP. In these facilities, more access to carrier screening, genetic counselling, and prenatal diagnostic services are made available.

In the present study, cultural beliefs, family pressures and religious issues were recognized as significant barriers. Such conclusions have been drawn in Pakistan and other south Asian nations where the family members and religious beliefs may play a role in the decision for prenatal testing (13, 15). Low utilization was also due to reluctance to invasive procedures, such as CVS.

The results of the present study suggest low awareness and low rate of prenatal molecular diagnosis among carrier couples. A small percentage of participants had been tested using a CVS test, a finding consistent with those of other low- and middle-income countries, but much lower than those of countries with established prevention programs.

The study underscores the importance of genetic counseling to enhance knowledge and use of prenatal diagnosis. Those who were counseled were more likely to have had prenatal testing. This is in line with the international literature that focuses genetic counseling as an important factor for the uptake of preventive genetic healthcare.

Conclusion

The present study reveals that Knowledge of PND among the couples carrying β-thalassemia is low in KPK Province. While attitudes toward prenatal testing are generally positive, the use of CVS-based prenatal diagnosis is limited. The main challenges faced were low awareness, financial problems, inaccessibility to health care services and sociocultural and religious factors. These results reveal a great gap between knowledge and practice and are also responsible for the occurrence of children with β-thalassemia major in the region.

REFERENCES

1. Cappellini M, Cohen A, Eleftheriou A, Piga A, Porter J, Taher A. Guidelines for the clinical management of thalassaemia. Guidelines for the Clinical Management of Thalassaemia 2nd Revised edition: Thalassaemia International Federation TIF; 2008.; 2014.
2. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bulletin of the World Health Organization. 2001;79(8):704-12.
3. Angastiniotis M, Modell B. Global epidemiology of hemoglobin disorders. Annals of the New York Academy of Sciences. 1998;850(1):251-69.
4. Ahmed S, Saleem M, Modell B, Petrou M. Screening extended families for genetic hemoglobin disorders in Pakistan. New England journal of medicine. 2002;347(15):1162-8.
5. Waheed Y, Shafi T, Safi SZ, Qadri I. Hepatitis C virus in Pakistan: a systematic review of prevalence, genotypes and risk factors. World journal of gastroenterology: WJG. 2009;15(45):5647.
6. Zaheer HA, Waheed U, Abdella YE, Konings F. Thalassemia in Pakistan: A forward-looking solution to a serious health issue. Global Journal of Transfusion Medicine. 2020;5(1):108-10.
7. Bittles AH, Black ML. Consanguinity, human evolution, and complex diseases. Proceedings of the National Academy of Sciences. 2010;107(suppl_1):1779-86.
8. Cao A, Kan YW. The prevention of thalassemia. Cold Spring Harbor perspectives in medicine. 2013;3(2):a011775.
9. Russo ML, Mahoney R, Driscoll DA, Al-Kouatly H. Genetic Screening and Diagnosis. Obstetrics: Normal and Problem Pregnancies E-Book. 2024:228.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

10. Old J. Screening and genetic diagnosis of haemoglobin disorders. Blood reviews. 2003;17(1):43-53.
11. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bulletin of the World Health Organization. 2008;86(6):480-7.
12. Samavat A, Modell B. Iranian national thalassaemia screening programme. Bmj. 2004;329(7475):1134-7.
13. Rehman AU, Ulhaq I, Khan W, Khan N, Ali Q, Ali A, et al. Awareness and practices regarding thalassemia among parents of transfusion dependent beta thalassemia patients registered with Hamza Foundation Peshawar, Pakistan. International Journal of Health Sciences. 2024;8(S1):241-56.
14. Memish ZA, Saeedi MY. Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and β-thalassemia in Saudi Arabia. Annals of Saudi medicine. 2011;31(3):229-35.
15. Colah R, Gorakshakar A, Nadkarni A. Global burden, distribution and prevention of β-thalassemias and hemoglobin E disorders. Expert Review of Hematology. 2010;3(1):103-17.
16. Canatan D. Thalassemias and hemoglobinopathies in Turkey. Hemoglobin. 2014;38(5):305-7.