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Spectrum of Mutations in Chorionic Villous Samples for Prenatal Diagnosis of Thalassemia in Patients Presenting at a Tertiary Care Diagnostic Centre

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ABSTRACT

Objective: To determine the spectrum of mutations in chorionic villous samples (CVS) for prenatal diagnosis of thalassemia in patients presenting at a tertiary care diagnostic centre.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Hematology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan, from Jul 2020 to Jan 2021.

Methodology: Chorionic villous sampling (CVS) was performed between the 12th and 16th weeks of pregnancy. A transabdominal technique was used to obtain placental specimen. The couples' blood samples were collected in vials coated with ethylene diamine tetra acetic acid (EDTA). Chelex technique was used to extract genomic DNA. For genetic analysis of mutations, the Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS-PCR) was used.

Results: A total of ninety-four women underwent chorionic villous sampling. After DNA analysis, β-thalassemia major was diagnosed in 18(18.9%), β-thalassemia trait found in 52(55.3%) cases and no mutation was detected in 24(25.5%) cases. Among the patients diagnosed with β-thalassemia major, 15(83.33%) were homozygous and remaining 3(33.33%) were compound heterozygous.

Conclusion: Prenatal chorionic villous sampling and molecular analysis is useful in identifying β-thalassemia and β-thalassemia trait during pregnancy.

Keywords: Beta Thalassemia, Chorionic Villi Sampling, Heterozygous, Homozygous, Molecular Analysis.

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INTRODUCTION

Thalassemia is a collection of diseases in which the production of healthy haemoglobin is slowed or stopped entirely due to a lack of globin chain synthesis.[1] Thalassemia is the most common autosomal recessive single gene haemoglobin synthesis disorder,[2] and is among the most frequently seen hereditary disorders in Pakistan, with a carrier prevalence of 5 to 7%. Every year, nearly 5000 homozygous thalassemia children are born in Pakistan, with consanguinity being a leading cause.[3]

Blood transfusions and iron chelating therapy are widely held treatments for β-thalassemia. Bone marrow transplant may also improve both survival rates and quality of life.[4] These types of management, however, are typically not easily affordable in un-industrialized nations where thalassemia is common and medical services are scarce. As a result, the most efficient and cost-effective way to address this issue is to avoid it.[5]

Recent advances in first trimester foetal-tissue screening have made it possible to diagnose several genetic abnormalities early in pregnancy, giving patients the choice of terminating the pregnancy if the foetus is impaired. Certain foetal genetic defects are diagnosed using CVS and amniocentesis.[6] CVS is now a well-known and well-established procedure for prenatal diagnosis of single gene disorders and chromosomal abnormalities.[7] The benefit of the procedure is that it allows for early diagnosis of disease (8-12 weeks), hence an option for timely cessation of pregnancy. Despite the fact that prenatal diagnosis of thalassemia by CVS is standard technique in many countries, it is still not widely used in Pakistan. Lack of knowledge, delay in obtaining advice, non-affordability and religious prejudice have all been reported as reasons for CVS sampling underutilization.[8,9]

The key indications for using molecular methods in thalassemia diagnosis include prenatal diagnosis, diagnosis of thalassemia in a previously transfused

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patient, identification of silent thalassemia alleles and differentiation between Hb-E/C and Hb-S/D/Lepore, among others.[10]

The present study was designed to see the spectrum of mutations in chorionic villous samples (CVS) for prenatal diagnosis of thalassemia.

METHODOLOGY

This cross-sectional study commenced after obtaining approval from the Institutional Ethical Review Committee [ref# FC-HEM17-35/READ-IRB121 /265] and was carried out from July 2020 to January 2021) in the Department of Hematology, Armed Forces Institute of Pathology Rawalpindi, Pakistan.

Inclusion Criteria: Couples with one or more children diagnosed with β-thalassemia major and both parents who had β-thalassemia minor were included.

Exclusion Criteria: Those without family history of β-thalassemia major were excluded.

Sample size was calculated using WHO calculator with prevalence of β- thalassaemia as 7%.[9] After signing written, informed consent 94 couples were included in the study using non-probability consecutive sampling.

Chorionic villous sampling (CVS) was performed between the 12th and 16th week of pregnancy. The placental sample was taken via the transabdominal approach after proper counselling. The couples' blood samples were collected in vials coated with ethylene diamine tetra acetic acid (EDTA). Chorionic villous samples were collected in [5-10] sterile normal saline placed in a disposable plastic or glass petri diss. The sample was carefully examined by the naked eye or a stereomicroscope to ensure that it contained sufficient chorionic villi. It was transferred with a Pasteur pipette, three to four adequately sized placental villi to a 1.5ml plastic eppendorf tube containing sterile normal saline. The tube was labeled and its cap was tightly secured in order to prevent from opening during transport. Genomic DNA was extracted using Chelex method. Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS-PCR) was utilized for genomic exploration of mutations.

Data was analyzed using Statistical Packages for the Social Sciences (SPSS) version 21.00. Qualitative variables were presented as frequencies and percentages and quantitative variables as Mean±SD.

RESULTS

A total of ninety-four (94) women underwent chorionic villous sampling procedure between the gestational age of 8-16 weeks with mean gestational age 12.5±3.0 weeks. Age range of the mothers was between the 18 to 40 years with mean age being 29.7±10.3 years. Most women (86.1%) had a child previously affected with β-thalassemia major. Seven women were pregnant for the first time.

Consanguineous marriage was noticed in 77(81.9%) and majority of these were first cousins (Table-I).

After DNA analysis, β-thlassaemia major was diagnosed in 18 (18.9%), β-thalassemia trait found in 52(55.3%) cases and no mutation was detected in 24(25.5%) cases (Table-II).

Molecular analysis revealed that among women detected with β-thalassemia trait, Intervening sequence (IVS) 1-5 was found in 17(32.69%) cases followed by Fr 89 in 16(30.79%), Fr 41-42-TTCT in 9(17.3%), Cd 5-CT in 4(7.9%), Fr 16-C in 3(5.7%), Cd 15 G-A in 2(3.8%) and IVS 1-1 G-T in 1(1.9%) case (Table-III).

Among patients diagnosed with β-thalassemia major, 15(83.33%) were homozygous and the remaining 3(33.33%) were heterozygous. In homozygous patients, IVS 1-5 G-C and Fr 8-9+ G was found in 4(26.6%) cases followed by Fr 41-42-TTCT in 3(20%), Del 619 bp in 2(13.3%), Fr 16-C and Cd 30 G-A in 1(6.6%) each (Table-IV).

In heterozygous patients, Fr 89+G/ Cd 15 G-A , Fr 41-42-TTCT/ Fr 8-9+G and Cd5-CT/Fr 8-9+G were seen in 1(33.3%) case each (Table-V).

Table-I: Patient's Demographic Details (n=94)

Characteristics	n (%)
Maternal age, Range (Mean+SD)	18-40 (29.7+ 10.3) years
Gestational age, Mean+ SD	12.5+ 3.0 weeks
Variable	n (%)
Primigravida Mother with second pregnancy >3rd pregnancy	8(8.5%) 17(18%) 69(73.4%)
Consanguineous marriage	77(81.9%)
1st Cousin	67(71.2%)
Previously effected child with beta thalassemia	81(86.1%)
CVS sampling at first pregnancy	7(7.4%)

Table-II: Chorionic Villous Samples (CVS) in β Thalassemia (n=94)

Entity	n (%)
Beta thalassemia major	18(18.9%)
Beta Thalassemia trait	52(55.3%)
No mutation detected	24(25.5%)

Table-III: Types of β Thalassemia Trait Mutation Detected on Molecular Analysis a of CVS samples (n=52)

Mutation	n (%)
IVS 1-5 G-C	17(32.69%)
Fr 8-9 + G	16(30.79%)
Fr 41-42-TTCT	09(17.3%)
Cd 5-CT	04(7.9%)
Cd 15 G-A	02(3.8%)
Fr 16-C	03(5.7%)
IVS 1-1 G-T	01(1.9%)

Table-IV: Homozygous Beta Thalassemia Major on Molecular Analysis of CVS samples. (n=15)

Mutation	n (%)
IVS 1-5 G-C	04(26.6%)
Fr 8-9+ G	04(26.6%)
Fr 41-42-TTCT	03(20%)
Del 619 bp	02(13.3%)
Fr 16-C	01(6.6%)
Cd 30 G-A	01(6.6%)

Table-V: Heterozygous Beta Thalassemia Major on Molecular Analysis of CVS samples.(n=03)

Mutation	n (%)
Fr 8-9+G/ Cd 15 G-A	01(33.3%)
Fr 41-42-TTCT/ Fr 8-9+G	01(33.3%)
Cd5-CT/Fr 8-9+G	01(33.3%)

DISCUSSION

There are approximately 50,000 patients of thalassemia major in Pakistan. There is a 25% probability of developing a foetus with thalassemia major in a carrier couple with an autosomal recessive disorder, a 25% chance of a normal fetus and a 50% chance of a foetus with. Prenatal diagnosis may help to determine this status.[11] The objective of the study was to determine the molecular spectrum of genetic mutations in CVS samples for prenatal diagnosis of thalassaemi.

Before 20 weeks of pregnancy, pregnant women of all ages should be given chromosomal abnormality screening and invasive diagnostic testing. CVS in the first trimester and amniocentesis in the second trimester are two diagnostic choices.[12] The introduction of first trimester screening programs has resulted in a decrease in the use of CVS, particularly among women aged 35 and above. However, since patients receive immediate results in 60.6% of cases, CVS use appears to be on the rise.[13] So far, over 200 mutations have been discovered in the globin chain synthesis, a quarter of which were discovered in a diverse Indo-Pak community. The majority of children born with this fatal disease are from developing countries.[14]

Another way to avoid disease is to discourage cousin marriages. Consanguinity was registered in 81.9% of the couples in our sample. These results are comparable with the studies conducted in Lahore and Rawalpindi in which consanguinity was stated in 82% and 86% of cases respectively. [15,16] Consanguinity was found in 59% of people in a Karachi survey which can be attributed to Karachi's more urbanized and literate population. In a family with β-thalassemia, it is more common to have genetic counselling, extended family screening, and prenatal diagnosis.[17]

In Pakistan, prenatal diagnosis and carrier detection are available, but their use is limited due to a lack of public awareness and the associated costs. In this study, 7.4% of the patients underwent for CVS procedure at first pregnancy. The findings are inconsistent with a study from Peshawar, in which it is stated that only 5.17% of the couples opted CVS at their first pregnancy.[3] However, the results are not in line with the studies from Islamabad and Karachi that quoted 38% and 39% of cases underwent CVS during their first pregnancies, respectively. The discrepancy in results may be due to different socio-economic status, educational status and ethnicity.[14,18]

Result of CVS showed that among ninety-four cases, β-thalassemia major was diagnosed in 18(18.9%), β-thalassemia trait was diagnosed in 52(55.3%) and 24(25.5%) cases were normal. Such findings were also noted in other studies.[19]

On molecular analysis, it was found that, IVS 1-5 was commonest mutation followed by Fr 8-9 in women detected with β-thalassemia trait on CVS. The findings are in comparison with the study conducted in southern Punjab, in which IVS 1-5(G-C) was the commonest mutation (42%) after Fr 8-9(+G), which was 40%20. On the other hand, a review of the spectrum of β-thalassemia mutations in Southern Punjab found that Fr-8-9(+G) was the most common mutation (30.1%), followed by IVS 1-5(G-C) which accounted for 29.2%.[21] This disparity is most likely due to a change in mutation frequencies. According to Indian studies, the range of β-thalassemia mutations should be evaluated on a regular basis.[22] In our study, IVS 1-5 and Fr 8-9 are more common in Punjabi's and Pathans. The results of present study are in agreement with other studies across the country, which shows predominance of Fr8-9 and IVS1-5 mutations in Punjabis and Pathans. For the Indian population, similar findings have been observed.[23] In studies conducted in the Mediterranean region, IVS1-1 (G-A),

IVS 1-110 (G-A), and IVS1-6 (T-C) mutations were found to be the most common β-thalassemia mutations, with IVS 1-101 (C-T) mutations being one of the most common silent β-thalassemia mutations. [24]

CONCLUSION

Prenatal chorionic villous sampling and molecular analysis is useful in identifying β-thalassemia and β-thalassemia trait during pregnancy.

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Authors Contributions:

Following authors have made substantial contributions to the manuscript as under:

AS & HMR: Conception, study design, drafting the manuscript, approval of the final version to be published.

FA & AM: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

SA & SZ: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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