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The Extent of Beta-Thalassemia Mutations in Couples Referred for Chorionic Villus Sampling: A Collaborative Single Center Cross-Sectional Study

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ABSTRACT

Background: Beta Thalassemia is the most pervasive single gene disorder of hemoglobin. As thalassemia is a preventable disease, its prevention must be our priority. Prenatal diagnosis in the form of chorionic villous sampling is a very important tool for its prevention. **Objective:** The purpose of our study is to ascertain the results of chorionic villous sampling performed for prenatal diagnosis of couples at a risk of having thalassemia major child and to scrutinize the extent of beta thalassemia mutations in accordance with region and ethnicity. **Study Design:** Retrospective cross-sectional study**. Settings:** Patients referred at regional Centre of Punjab thalassemia prevention program (PTPP) of Sahiwal Teaching Hospital. **Duration:** From October, 2020 to August, 2022. **Methods:** After taking informed consent, chorionic villous sampling (CVS) was done of 75 women at gestational age of 11-15 weeks, referred to regional center of Punjab thalassemia prevention program of Sahiwal Teaching Hospital. Following Sops of sample taking and transportation, genetic analysis of sample was done at PTPP, Lahore. **Results:** The results of CVS in our study showed that 21.4% turned out to be unremarkable (no mutation detected). 58.6 % were found to have thalassemia trait/carrier while 20.0% were positive for mutation of thalassemia major. The most common mutation in our study detected in thalassemia major fetuses was Fr 8-9 (+G) followed by IVS I-5 (G-C) and Cd 30 (G-C). Although region wise distribution of mutations remained statically non-significant in our study. **Conclusion:** We concluded that certain mutations of thalassemia major are common in region that must be included in screening panel.

Keywords: Chorionic villus sampling, Beta thalassemia, Spectrum of mutations, Prenatal diagnosis.

INTRODUCTION

Beta Thalassemia is the most pervasive single gene disorder of hemoglobin. It targets Mediterranean region including Southeast Asia and Middle East.[1] Approximately 70,000 births of b-thalassemia are noted each year in the world. Carrier rate (thalassemia trait) in Pakistan is estimated to be about 9.8 million. About 5000 – 9000 births are estimated annually in Pakistan.[2] As the trend of cousin marriage in high in our country, this contributes to the high prevalence of the disease in Pakistan. Moreover, marriages in same ethnic groups, large population size and lack of awareness also accounts for the high rate of thalassemia in our setup.[3]

Quality of life of is markedly handicapped in children suffering from beta-thalassemia major.[4] As thalassemia is a preventable disease, so our more focus must be on prevention rather than on treatment to avoid up-scaled bone marrow transplant that is the ultimate cure for thalassemia children.[5] As far as regional distribution is concerned, certain mutation are more common in Pakistan. This depends on ethnicity as well.[6] This data facilitates the screening program regarding adequate use of resources to screen only those mutation firstly that are more common in our region. Therefore, it is quite beneficial to analyze regional and ethnicity wise date to prevent wastage of resources. This would make the screening and prevention program feasible as well as cost effective.

Sahiwal is a densely populated area of Punjab between Lahore and Multan. It is the 21st largest city of Pakistan by population. Regional center of PTPP in Sahiwal was established in 2020 and till now CVS of 70 couples who were at a risk of having thalassemia major

child has been performed. These include cases from Okara and Pakpattan District in addition to residents of Sahiwal. This study will elaborate the results of CVS and type of commonly detected mutations in this region. This data will aid in making screening program targeted and cost- effective for better prevention of thalassemia major in Pakistan.

METHODS

This retrospective cross-sectional study was conducted on couples having thalassemia trait referred for CVS to regional Centre of Punjab thalassemia prevention program (PTPP) at Sahiwal Teaching Hospital from October, 2020 to August, 2022. Institutional ethical review board granted approval for the study vide letter No. 87/SLMC/SWL, dated: 07-06-2021. 70 pregnant women at a gestational age of 11 – 15 weeks, where both partners of couple having thalassemia trait and one or more child suffering from thalassemia major were included in the study. Couples having co-morbidities like cardiovascular, renal or any other hematological disorders were excluded. After taking informed consent, chorionic villous sampling (CVS) was performed via trans-abdominal approach. Placental tissue along with 2 cc of blood in ethylenediaminetetraacetic acid (EDTA) vials were taken following sops and sent to DNA lab of PTPP in Lahore for genetic analysis.

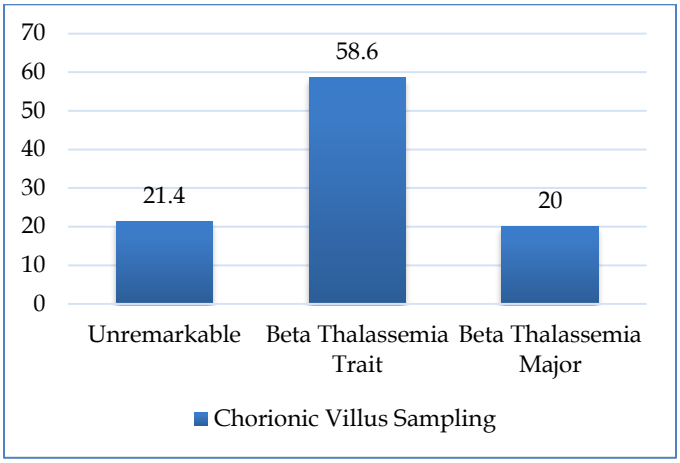
RESULTS

We analyzed 70 samples in total for results of CVS. Mean age of the patients in our study is found to be 28.45 ± 5.02. Median Gestational Age is found to be 13.5 ± 0.95 at the time of CVS sampling. The results of CVS in our study showed that 15 cases (21.4%) turned out to be unremarkable (no mutation detected). 41 fetuses (58.6%) were found to have thalassemia trait/carrier while 14 cases (20.0%) were positive for mutation of thalassemia major. Figure 1. and Table 1. Among these 14 thalassemia major cases, 04 were compound heterozygotes and rest 10 cases were homozygous for the detected mutation.

Table 1: Results of Chorionic Villus Sampling

Mutation Detected	Frequency	Percent	Valid Percent	Cumulative Percent
Yes	14	20.0	20.0	20.0
No	56	80.0	80.0	100.0
Total	70	100.0	100.0	

Figure 1: Results of Chorionic Villus Sampling



Our results revealed that the most common mutation detected in thalassemia major fetuses was Fr 8-9(+G) followed by IVS I-5 (G-C) and Cd 30 (G-C). Table 2

Although region wise distribution of mutations remained statically non-significant in our study. Table 3

Table 2: Types of mutations detected

Type of Mutation	Frequency	Percent	Valid Percent	Cumulative Percent
B-Globin Allele 1 ->1 VS -15(G-C) B-Globin Allele 2 ->Fr 4142 (-TTCT)	1	1.4	1.4	1.4
B-Globin Alle1-> Fr 8-9(+G) B-Globin Alle2-> Fr 8-9(+G)	5	7.1	7.1	8.6
B-Globin Alle1-> Cd 30 (G-C) B-Globin Alle2-> Cd 30 (G-C)	3	4.3	4.3	12.9
B-Globin Alle1-> IVS I-5 (G-C) B-Globin Alle2-> Fr4142 (-TTCT)	3	4.3	4.3	17.1
B-Globin Alle1-> Cd 15 (G-A) B-Globin Alle2-> Cd 15 (G-A)	2	2.9	2.9	20.0
No Mutation Detected	56	80.0	80.0	100.0
Total	70	100.0	100.0	

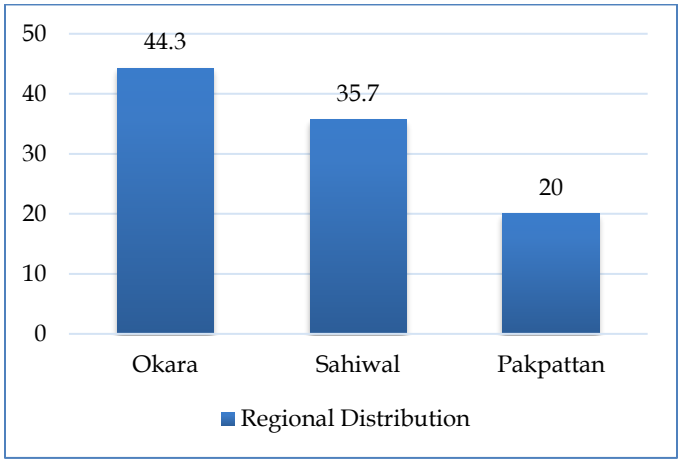
Table 3: Regional Distribution of Different Mutations

Type of Mutation	Sahiwal	Okara	Pakpattan	Total	P-Value*
B-Globin Allele 1 ->1 VS -15(G-C) B-Globin Allele 2 ->Fr 4142 (-TTCT)	1	0	0	1	0.967
B-Globin Alle1-> Fr 8-9(+G) B-Globin Alle2-> Fr 8-9(+G)	1	3	1	5	
B-Globin Alle1-> Cd 30 (G-C) B-Globin Alle2-> Cd 30 (G-C)	1	2	0	3	
B-Globin Alle1-> IVS I-5 (G-C) B-Globin Alle2-> Fr4142 (-TTCT)	2	1	0	3	
B-Globin Alle1-> Cd 15 (G-A) B-Globin Alle2-> Cd 15 (G-A)	1	1	0	2	
No Mutation Detected	19	24	13	56	
Total	25	31	14	70	

Statically Significant P- Value < 0.05*

As far as regional wise distribution is concerned, 35.7 % cases were of Sahiwal region while 44.3 % and 20.0 % were from Okara and Pakpattan respectively. Figure 2

Figure 2: Regional Distribution



DISCUSSION

A carrier couple of beta thalassemia trait has a 25 % chance of having an unaffected fetus, 50 % chance of fetus with thalassemia trait and 25 % chance of having a fetus with thalassemia major.[7] Our study showed unremarkable results in 21.4% of cases while 58.6 % cases were thalassemia trait/carrier and 20.0% cases were of thalassemia major.

Our results closely correlate with a study conducted in Peshawar which showed thalassemia carrier rate 42.8 %, thalassemia major 21.4 % while no mutation detected in 31.4 %.[8] A study conducted in south eastern China by Huang H et al showed prevalence of thalassemia trait in their study was 43.49% while of thalassemia major was 26.05% which also closely mimic our results.[9]

The most common mutation detected in our study was Fr 8-9(+G) followed by IVS I-5 (G-C) and Cd 30 (G-C). Similar findings were shown by a study conducted in Bahawalpur by Zaffar U et al.[10] These findings were also endorsed by a study of Khateeb B et al.[11] Baig SM et al's study also showed the most common mutation among Punjabis was Fr 8-9 followed by IVS I-5.[12]

The knowledge about certain mutations common in specific ethnic group has a very beneficial role in prevention of disease at all levels. However, as Sahiwal is a main region between Multan and Lahore and covers mainly regions like Okara and Pakpattan, the major ethnicity of population in this area is Punjabi. The limitation of our study is that we could not cover other ethnic groups in our study. Also, the sample size was limited. So, more studies with large sample size that can include all ethnic groups of Pakistan are needed for detailed analysis of this spectrum.

CONCLUSION

This extent of thalassemia major mutations suggests that these mutations are common in our region. Moreover, other mutations that are not appearing in our population, their primers can be omitted from diagnostic kits to make the cost-effective approach for prevention and diagnosis of thalassemia major in our country.

LIMITATIONS

In our study, we could not include subjects from all ethnic groups. The sample size of our study was also limited.

SUGGESTIONS / RECOMMENDATIONS

More studies involving large number of subjects and including all ethnic groups are needed for detailed analysis of common mutations causing thalassemia in our region.

CONFLICT OF INTEREST / DISCLOSURE

Nil.

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