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STUDY OF THE EPIDEMIOLOGY AND MOLECULAR ETIOLOGY OF SELECTIVE PATIENTS OF B-THALASSEMIA IN VIDARBHA REGION

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ABSTRACT

Background: Thalassemia is a group of genetic disorders characterized by quantitative defects in globin chain synthesis with subsequent absence or decrease of haemoglobin production leading to variable degrees of microcytic anaemia. It is commonly found in people of Mediterranean, African, Middle Eastern, Indian, Chinese, or Southeast Asian origin. β-thalassaemia is an autosomal recessive single gene disorder characterized by reduced β+ or β0. B globin chain synthesis leads to reduced haemoglobin A (HbA) synthesis. By the advance, PCR based DNA diagnostic techniques; it is now possible to offer diagnosis of thalassemia using extracted blood DNA. **Aim and Objective:** This study was done with an aim to evaluate the epidemiology and molecular etiology of β-Thalassemia in Vidarbha region. **Materials & Methods:** 30 blood samples were collected from Thalassemia patients and DNA was extracted from peripheral blood lymphocytes of the patient using Spin column method of blood DNA extraction kit (Vivantis™ GF-1 Blood DNA Extraction Kit). The DNA thus obtained was processed by a qualitative conventional PCR reaction to detect the amplification of 4 different genes (4 mutations), using 7 specific primers followed by agarose gel electrophoresis of the amplicons and visualized by ethidium bromide. We also collect data regarding the onset of diseases along with some socioeconomic questionnaire. **Result:** 30 blood samples were collected from β-thalassemia carriers (minor) from Vidarbha Region. Out of which four common β-thalassemia mutations, IVS I-nt 5(G-C), IVS I-nt 1(G-T), Co 8/9 (+G) and Co 41/42 (-CTT) were found in random population of Vidarbha Region, in 43%, 23%, 17% & 10% respectively. **Conclusion**: These observations might help in forming β- thalassemia database of the region which may useful for genetic counseling and prenatal diagnosis.

KEYWORDS: β-thalassemia, epidemiology, molecular etiology, PCR, mutations analysis.

INTRODUCTION

Thalassemia is a group of genetic disorders characterized by quantitative defects in globin chain synthesis with subsequent absence or decrease of haemoglobin production leading to variable degrees of microcytic anaemia. It is commonly found in people of Mediterranean, African, Middle Eastern, Indian, Chinese, or Southeast Asian origin. β-thalassaemia is an autosomal recessive single gene disorder characterized by reduced β+ or β0.[1] β-globin chain synthesis leading to reduced haemoglobin A (HbA) synthesis. By the advance, PCR based DNA diagnostic techniques; it is now possible to offer diagnosis of thalassemia using extracted blood DNA.

β-Thalassemia major (β-TM) is the most severe and important type causing severe transfusion-dependent anemia with reduced life expectancy if untreated properly.[2] More than 95% of the genetic disorders responsible for β-globin genes are base substitution mutations, while a minority of the mutations corresponds to gene deletion.[3] The severity of the clinical syndrome of β-Thalassemia depends on the type of mutation in the β-globin gene. More than 400 different mutations have been reported and identified in the β-globin gene which is responsible for the development of the β-Thalassemia.[4] In addition to the direct effects of altered or reduced β-globin synthesis, many of the clinical features of this disorder appear to be consequence of the resulting cytotoxic buildup of free α-globin chains.[5,6]

Excess of unbound α-globin chains gets precipitated in erythroblast precursors in the bone marrow causes premature hemolysis and ineffective erythropoiesis[7] β-Thalassemia major patients are usually treated by blood transfusion. Humans have a very limited ability to excrete iron, hence regular blood transfusions inevitably

lead to iron overload,[8] which is the most relevant problem related with transfusion therapy.[9]

Many communities in India have a high prevalence of the β-thalassaemia gene. It varies between 1% and 17% with a mean prevalence of about 3.3%. Recent multicentric study among school children aged 11-18 years in Mumbai, New Delhi and Calcutta by the Task Force of the Indian Council of Medical Research (ICMR), New Delhi, showed a β-thalassaemia carrier rate of 2.6%, 5.5% and 10.2%, respectively, in these three cities.

In India, several studies were conducted to identify the types of mutations that cause thalassemia depending on the geographical distribution of these mutations in different parts of the country.[10,11,12] However, these studies didn't focus on mutations causing thalassemia in Vidarbha region. Henceforth, the current study was done with an aim to evaluate the epidemiology and molecular etiology of Thalassemia in Vidarbha region.

MATERIALS AND METHODS

Patient Selection

Total 30 cases of β- thalassemia in Vidarbha Region were studied for the types of β- thalassemia mutations, the informed consent obtained from patients and their parents. The diseases were excluded from the study. The healthy participants were taken as control.

Sample Collection

The 2ml peripheral blood samples were collected under sterile conditions from patients by venipuncture into EDTA tubes. Total 30 peripheral blood samples were collected for DNA extraction.

PCR Analysis

The DNA was extracted from using Spin column method of blood DNA extraction kit (Vivantis^{TM} GF-1 Blood DNA Extraction Kit). The PCR was carried out in a total volume of 25µl reaction mixture, containing 5µl of extracted DNA, 12.5µl of 2x PCR Master mix, 1µl of Primer mix,1ul of internal control and 4µl of nuclease free water. Cycling was carried out on the thermal cycler with initial denaturation at 94°c for 5 min followed by 30 cycles of denaturation at 94°c for 1min, annealing at 66 °C for 1min, extension at 72°c for 1.5 min followed by final extension at 72 °C for 10mins and then cooling at 4 °C for 10 min. Amplified PCR products were separated on 1.5% agarose gel. 100 bp DNA Ladder was used as a molecular size standard. The gel were stained with Ethidium bromide and photographed under UV light in BioRad Gel-doc system

RESULT

30 blood samples were collected from β-thalassemia patients from Vidarbha region. Out of which four common β-thalassemia mutations, IVS I-nt 5(G-C) was seen in 13 (43%) samples, IVS I-nt 1(G-T) was seen in 7(23%) samples, Co 8/9 (+G) was seen in 5(17%) samples, Co 41/42 (-CTT) was seen in 3 (10%) samples where as 2 (43%) samples shows nonspecific amplification.

Table 1: β-thalassemia mutations.

Sr. No	Mutation	Mutations(30)	Percentage
1	IVS I-nt 5(G-C)	13	43.33%
2	IVS I-nt 1(G-T)	7	23.33%
3	Co 8/9 (+G)	5	16.66%
4	Co 41/42 (-CTT)	3	10%
5	Nonspecific	2	6.66%

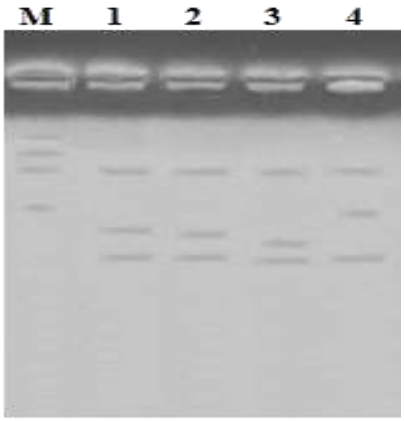

Fig. 1: β-thalassemia mutations, M: Marker, Lane1: IVS I-nt 5(G-C), Lane 2: IVS I-nt 1(G-T), Lane 3: Co 8/9 (+G), Lane 4: Co 41/42 (-CTT).

DISCUSSION

All molecular diagnostic technique has various limitations. Molecular analysis is significantly accurate, but it can be expensive and time-consuming without proper planning. Allele-specific oligonucleotide probes (ASO) dot blot hybridization is reliable and easy for populations predominated by only one or two mutations,[13] but becomes time-consuming because it requires separate hybridization and washing steps to screen for multiple mutations.[14]

Nowadays, more sophisticated techniques, such as real-time PCR, high resolution melting analysis (HRM), and oligonucleotide microarray analysis, have been reported. Allele-specific Q-primer real time-PCR[15] is cheap, rapid and high-throughput, but limited to the screening of several common mutations only. The bead-based biosensor described by Ng *et al.*[16] is rapid, high-throughput and more sensitive, but the fabrication process can be complicated and costly. In our study, we had used more simple and less expensive qualitative conventional PCR reaction to detect the amplification of 4 different genes (4 mutations), using 7 specific primers.

In Southeast Asia carrier screening revealed a high frequency of β-thalassaemia ranging from 3% to 10% in

Indonesia, , 0.93% in Singapore, 1.5% in Vietnam, 0.5% to 1.5% in Myanmar and 3% to 9% in Thailand.[17] We found a high frequency of carriers with Cd 26 (G–A), IVS1–5 (G–C), IVS 1–1 (G–T), and Cd 41/42 (–TTCT) among the Malays. Previously, only Cd 26 (G–A) and IVS1–5 (G–C) were reported as common among the Malays.[18,19] The high frequencies of Cd 26 (G–A) HbE and IVS 1–5 (G–C) were similar to finding in Indonesia.[20] Hassan *et al.* recorded five times higher frequencies of Cd 26 (G–A) HbE carriers (23%) as compared to previous micromapping studies that showed 4% among the Malays.[21] In total, 29% (54/185) of patients were found with Cd 26 (G–A) HbE, including 11 compound heterozygous Cd 26 (G–A) HbE/β-thalassaemia and 73.3% (11/15) homozygous for Cd 26 (G–A) HbE.[22,23,24]

In Chinese population, Cd 41/42 (–TTCT), IVS2–654 (C–T), and –28 (A–G) mutations accounted for 88% (24/27) of the mutations. Thong *et al*.[25] reported a high frequency of similar mutations among the Chinese. Interestingly, we found a 22-year-old Kedayan with Cd 8/9 (+G) and Poly A (A–G) who was diagnosed as β-thalassaemia major, and who had undergone regular blood transfusions since he was 8-months old; and a Chinese-Malay descendant patient with heterozygous IVS 2–654 (C–T) mutations.

Prevalence of β-thalassaemia trait was 4.4%. Hindu, Muslim and Jain communities had comparable prevalence. In a collaborative study of Indian council of medical research (ICMR) 2.5% Sunni and 2.8% Shiya Muslims in Mumbai had β-thalassaemia trait whereas in Delhi 1.7% β-thalassaemia trait was reported in Sunni Muslim.[26] In Mumbai and Delhi Jain community had 3.3% and 4.8% β-thalassaemia trait prevalence respectively.

The Gamit, Chaudhary, Vasava tribals and Lohana, Sindhi non-tribal communities had greater than 10% prevalence of β-thalassaemia trait. Vyas *et al*.[27] investigated Gamit tribals from Surat district for SCT and SCD, but there are no reports of β-thalassaemia trait studies in them. Cutchhi, Halai and Sindhi Lohanas have been studied by Bhatia *et al*.[28] and 10.7%, 17.2% and 6.8% prevalence of β-thalassaemia trait respectively has been reported in them. Mulchandani *et al.*[29] have reported 16.81% prevalence of β-thalassaemia trait in Sindhis which is higher than that reported. Prajapati, Ghanchi, Mahiyavanshi (non-tribal) and Rohit (tribal) communities also had high incidence ranging from 6.2 to 6.9%. Gamit, Chaudhary, Vasava, Mahyavanshi and Rohit had the high prevalence of β-thalassaemia trait. Overall prevalence of β-thalassaemia trait was 4.3% in Patel caste, with highest prevalence in Kukana followed by Kachhiya and Leva Patel.

In our study four common β- thalassemia mutations, IVS I-nt 5(G-C) was seen in 43% samples, IVS I-nt 1(G-T) was seen in 23% samples, Co 8/9 (+G) was seen in 17% samples, Co 41/42 (-CTT) was seen in 10% samples where as 3% samples shows nonspecific amplification.

Literature suggests that iron deficiency is less common in β-thalassaemia trait.[30,31] In the present study over all incidence of mild to moderate anemia was 75.1% in β-thalassaemia trait, 42.8% in SCT and 31.1% in normal subjects, negative for any hemoglobinopathy. In Sindhi community also anemia is more common in β-thalassaemia trait male and females compared to non- β-thalassaemia trait individuals.[32] However as anemia is not due to iron deficiency iron therapy is ineffective in these cases.

CONCLUSION

In conclusion, we have reported the used of inexpensive and easily interpreted techniques to identify 4 common mutations. These observations might help in forming β-thalassemia database of the region which may be useful for genetic counseling and prenatal diagnosis. We hope further screening will facilitate the genetic counselling of transfusion-dependent children, and pre-marital and pregnancy planning.

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