

## Original Research Article

# Prevalence of beta thalassemia mutations in population of Gujarat using amplification-refractory mutation system–polymerase chain reaction

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### ABSTRACT

**Background:** Beta thalassemia is the most common genetic disorder in India. Its trait, coinheritance and mutations vary from mild to severe condition, resulting in thalassemia minor (heterozygous), intermediate and major depending upon many factors. The objective of this study was to find out the prevalence rate and the carrier of beta thalassemia in population of Gujarat using molecular genetic analysis of beta thalassemia patients by targeted mutation assay (ARMS-PCR).

**Methods:** A total 105 samples for beta thalassemia were analysed for IVS 1-5 (G→C) and CD 15 (G→A) mutations. These two common mutations of thalassemia in Gujarat were carried out using amplification refractory mutation system–polymerase chain reaction (ARMS-PCR) and gel electrophoresis method.

**Results:** A total 105 samples referred to us for molecular genetic analysis. The occurrence of positive mutations of IVS 1-5 (G→C) and CD 15 (G→A) were found in 48 and 15 samples respectively. The rest were negatives.

**Conclusions:** Present study concludes that the prevalence rate of Beta thalassemia was widespread among the Gujarat population. The identification of IVS 1-5 (G→C) and CD 15 (G→A) mutations was carried out. The analysis revealed that, mutational patterns of IVS 1-5 (G→C) was the most frequent among other mutations in Gujarat region.

**Keywords:** Haemoglobinopathies, Thalassemia, ARMS-PCR, IVS 1-5 (G→C), CD 15 (G→A) mutations

### INTRODUCTION

Thalassemia is one of the most commonly inherited genetic disorders worldwide and even in India. Certain communities in India have a high predisposition to beta-thalassemia. To offer prenatal diagnosis and to prevent the birth of an affected child, mutation testing in clinically diagnosed beta-thalassemia patients/carriers is a prerequisite. Globally, more than 200 different mutations have been identified in the Beta globin gene, which are responsible for the development of the beta thalassemia. Most types of beta thalassemia are due to point

mutations, and large deletion mutations are found in rare cases.<sup>1</sup>

Hemoglobin disorders are the lethal inherited disorder worldwide and are very common. They have been reported commonly in population of tropical Africa, Asia and the Mediterranean region. Hemoglobin disorder causes death of 3.4% children who are below 5 years of age. Internationally, about 7% of women are carrier of  $\beta$  or  $\alpha$  thalassemia, or hemoglobin S, C, D or E, and over 1% of parents are at risk.<sup>2</sup>

Hemoglobinopathies are causing various medical problems and ultimately health burden in India. The frequency of Hemoglobinopathies in India is 42%. With a population above one billion and a considerable birth rate of 28 per thousand. There are about 42 million carriers and over 12,000 infants born each year with Hemoglobinopathies.<sup>3</sup>

The frequency to carry hemoglobinopathy ranges from 3-17% India amongst various population groups. Beta thalassemia is the common single-gene disease in the India.<sup>4</sup> 10% children suffering from thalassemia globally are born in India every year. Some communities such as Sindhi, Gujarati, Punjabi, and Bengali, are prone to beta thalassemia, with a variation from 1 to 17%.<sup>5</sup>

Hemoglobinopathies are more common in Gujarat compared to other Indian states, it was estimated as 12% incidence of major hemoglobinopathy traits in Gujarat.<sup>6</sup> The Gujarat comprises numerous castes and tribal groups, each revealing different genetic traits. Several studies have revealed high prevalence of beta thalassemia trait in some caste groups in Gujarat.<sup>7</sup>

Present study is an attempt to screen for Beta thalassemia trait in Gujarat population with an objective to investigate some of the communities which have never been screened for beta thalassemia.

## METHODS

The blood samples from the couples, when available were collected with EDTA as anticoagulant for DNA extraction. The beta thalassemia mutations of the parents/couples were analysed using the ARMS-PCR technique.

A total of 105 samples from different regions of South Gujarat, India were screened for beta thalassemia from March 2018 to March 2019. The genomic DNA was isolated using column based genomic DNA extraction kit (Qiasm DNA mini kit). ARMS-PCR was done to identify the beta thalassemia mutation pattern IVS 1-5 (G→C) and CD 15 (G→A). Primer sets which were selected for ARMS analysis of mutations for beta thalassemia.

A 25µl PCR reaction mix was prepared by adding Distilled water, 10X buffer containing 15mM MgCl<sub>2</sub>, dNTPs, Internal Control Primers (Forward & Reverse), Test primers (Mutant primer, Normal Primer, Common primer), Taq DNA Polymerase and the DNA sample.

Amplification is done with 1 cycle Initial denaturation at 93°C for 5 mins, 25 cycles each of denaturation at 93°C for 1 minute and annealing at 66°C for 2 mins, 1 cycle each of extension at 72°C for 1 minutes and final extension at 72°C for 1 minutes and finally a 10°C as holding temperature.

The ARMS-PCR products and the ladder marker are resolved by electrophoresis. DNA bands are visualized using Gel documentation system and the pattern of bands obtained on the gel are observed by comparing both the mutant and normal set according to the DNA products size with that of the DNA ladder to detect the mutations.

## Inclusion

The beta thalassemia patients who showed the value of MCV <80 fl and MCH <27 pg with relatively high RBC count were included. The level of HbA<sub>2</sub> was more than 4.0% were included.

## Exclusion

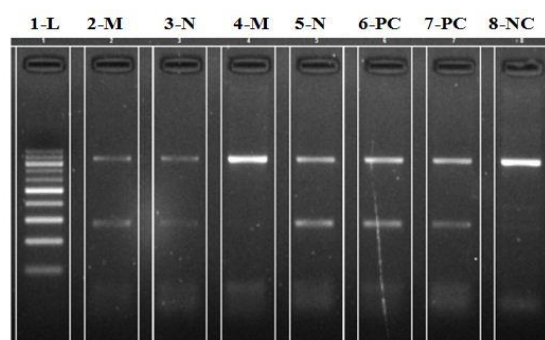
The others variants of hemoglobinopathy were excluded from the present study.

## RESULTS

Molecular study was carried out in the samples which were positive for beta thalassemia heterozygous and carrier. The analyses revealed that the mutational pattern of IVS 1-5 (G→C) and CD 15 (G→A) were most common mutations.

In all successful ARMS-PCR reactions, the internal control product band was observed, which was considered as a mandatory sign of successful reaction upon gel electrophoresis.

Out of the 105 samples (Table 1) studied for beta thalassemia mutational pattern, 48 (45.7%) samples showed, the mutational pattern IVS 1-5 (G→C) (Figure 1), and 15 (14.2%) samples were recorded with mutational pattern of CD 15 (G→A) (Figure 2).

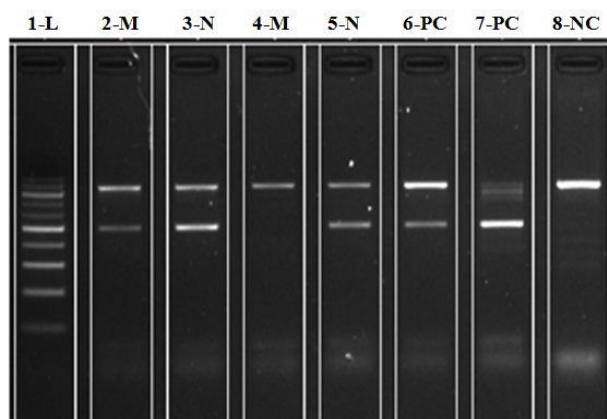


**Figure 1: Gel picture showing IVS 1-5 (G→C) mutation.**

- Lane 1-100bp ladder
- Lane 2-Sample-1-IVS1/5 M
- Lane 3-Sample-1-IVS1/5 N
- Lane 4-Sample-2-IVS1/5 M
- Lane 5-Sample-2-IVS1/5 N
- Lane 6-PC-IVS1/5 M
- Lane 7-PC-IVS1/5 N
- Lane 8-NC-IVS1/5 N

**Table 1: Prevalence of beta thalassemia mutation observed by postnatal diagnosis.**

Mutations	Beta thalassemia positive	Beta thalassemia carrier (%)
IVS 1-5 (G→C)	48	45.7
CD 15 (G→A)	15	14.2
<b>Total number of positive samples for IVS 1-5(G&gt;C) and CD 15 (G&gt;A)</b>	63	59.9

**Figure 2: Gel picture showing CD 15 (G→A) mutation.**

- Lane 1-100bp ladder
- Lane 2-Sample-5-**CD15 M**
- Lane 3-Sample-5-**CD15 N**
- Lane 4-Sample-8-**CD15 M**
- Lane 5-Sample-8-**CD15 N**
- Lane 6-PC-**CD15 M**
- Lane 7-PC-**CD15 N**
- Lane 8-NC-**CD15 N**

## DISCUSSION

Beta thalassemia is an inherited and autosomal recessive trait and is not linked by sex chromosomes. Affected patients always carry a heavy burden of morbidity and early death. Affected children require frequent transfusions and iron chelation therapy and measurement of liver iron concentration is necessary for such disorders of blood, which is beyond the affording capacity of the patients.<sup>8</sup>

The thalassemia poses an increasing burden for health care services in many Asian countries including India. In order to conserve rare resources, it is essential to determine the reasons for remarkable phenotypic heterogeneity and natural history of these disorders so that the most cost effective methods for their control and management can be established.<sup>9</sup>

It is one of the most common single gene disorders with >400,000 new born affected per year worldwide. The

incidence of Beta thalassemia trait in India ranges from 3.5 to 15%. Every year more than 10,000 children with thalassemia major are born in India. Affected individuals also have a shortage of red blood cells (anaemia), which can cause pale skin, weakness, fatigue, and more serious complications.<sup>10</sup>

The Indian population comprises numerous castes and tribal groups, each revealing different genetic traits. Several studies in the literature have reported that Gujarat has higher frequency of Beta thalassemia. Therefore prospective studies are essential to identify high risk communities. Premarital screening not only gives prevalence in different caste groups but also helps in counseling and prenatal diagnosis programs for prevention of birth of an affected child. In this study, the molecular bases of beta thalassemia have been investigated among individuals from the Gujarat. IVS 1-5 (G→C) and CD-15(G→A) beta thalassemia mutation was encountered among the investigated individuals accounting for 45.7 % and 14.2%. The study correlates with previous study by where the most common mutation identified among Asian Indians were IVS 1-5 (G→C) and CD-15(G→A).<sup>11</sup>

Earlier reports suggest that IVS 1-5 (G→C) is the most common mutation in the Indian population. However, in the Eastern region of India, a high frequency of IVS 1-5 (G→C) (72%) was reported<sup>11</sup>. In another study it was reported that the prevalence of IVS 1-5 (G→C) varied from 44.8% in the North to 71.4% in the East region of India. With reference to western region the prevalence rate of IVS 1-5 (G→C) was recorded to be 54.7 %.<sup>12</sup>

In present study showed that most common beta thalassemia mutation, IVS 1-5 (G→C) and CD 15(G→A) is being analyzed here to get information whether this common mutation is prevailing among the domicile of Gujarat or not. The study included characterization of IVS 1-5 (G→C) and CD 15 (G→A) mutations by ARMS-PCR. The total 105 samples referred for molecular genetic analysis, the occurrence of IVS 1-5 (G→C) and CD 15 (G→A) were observed as 48 (45.7%) and 15 (14.2%) respectively for samples having beta thalassemia heterozygous. The preliminary information regarding the mutational pattern is important for establishing prenatal diagnosis programmers.

This study will also suggest that to know the burden of disease in Gujarat and should help to plan screening, prevention and treatment services in that region where they are most needed. It also emphasizes the importance of local micro-mapping of population subgroups to determine the overall health burden of a common genetic disease.

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