



ISSN Print: 2394-7500  
ISSN Online: 2394-5869  
Impact Factor: 5.2  
IJAR 2015; 1(8): 273-275  
www.allresearchjournal.com  
Received: 17-05-2015  
Accepted: 20-06-2015

**Sangita S Kunjwani**  
Shri Shivaji College of Arts,  
Commerce & Science, Akola  
(MS)

**Zia H Khan**  
Shri Shivaji College of Arts,  
Commerce & Science, Akola  
(MS)

## Spectrum of $\beta$ -Thalassemia Mutations in high risk Sindhi community of Akola region

**Sangita S Kunjwani, Zia H Khan**

### Abstract

$\beta$ -thalassemia is a group of heterogeneous recessive disorders common in many parts of World and one of major haemoglobinopathy of wide occurrence in the Indian sub-continent characterized by reduced or absent  $\beta$ -globin chain production. It is distributed to different degrees in different sub-populations. The treatment is quite expensive and counseling seems to be the only way for controlling it. In this report genetic analysis of few common mutations was carried out by PCR technique. 50 blood samples were collected from  $\beta$ -thalassemia carriers (minor) from Akola region. Out of five common  $\beta$ -thalassemia mutations, IVS 1-nt 5(G-C), IVS 1-nt 1(G-T), Co 8/9 (+G) and Co 41/42 (-CTT) were found in Sindhi population of Akola region in 66%, 16%, 10% & 2% respectively. Such type of observations might help in forming the basis for comprehensive diagnostic database that would not only be useful for genetic counseling but also for prenatal diagnosis.

**Keywords:**  $\beta$ -thalassemia, mutational analysis, PCR

### 1. Introduction

$\beta$ -Thalassemia is a highly prevalent autosomal recessive blood disorder characterized by the reduced or absent expression of the  $\beta$ -globin gene (HBB) on the short arm of chromosome no. 11. HBB gene of hemoglobin encodes the beta-globin chains of hemoglobin an oxygen carrying protein composed of two alpha and two beta chain subunits found within red blood cells. The gene has three exons and two introns involved in beta thalassemia pathogenesis.

Inheritance of a single allele results in the beta-thalassemia minor (Carrier) while two beta-globin mutations, one from each parent, causes clinical severity of the  $\beta$ -thalassemia major or Cooley's anemia. The severe form leads to dependency on blood transfusion for long-life along with iron chelating therapy to combat iron overload. Expensive treatment imposes a significant burden on the available resources for health care and the disease may have dramatically psychological effects on families. However  $\beta$ -thalassemia major can lead to decreased life expectancy, if left untreated. Therefore  $\beta$ -thalassemia mutations detection, genetic counseling and prenatal diagnosis programmes play an important role in the prevention of the disease. (N Saleh-Gohari & MR Bazrafshani, 2010) [8]

Mutations in the HBB gene lead to an altered beta-globin chain, resulting in structural change of the protein conformation of hemoglobin (S.A. Harisha *et al.* 2014) [11]. According to the regularly updated online database of human hemoglobin variants and thalassemias on the Globin gene server website (<http://globin.cse.psu.edu>). More than 200 different mutations have been reported to date and each ethnic population has its own cluster of common mutations. Most  $\beta$ -thalassemias are caused by a point mutation, minor deletion, or insertion in the  $\beta$ -globin gene which result in reduced or lack of synthesis of the  $\beta$ -globin chain of hemoglobin. (Q-H Mo *et al.* 2005) [10]

There are few  $\beta$ -thalassemia mutations which are common in India. Twenty-two  $\beta$ -thalassemia mutations have been documented by various authors in Indian patients with  $\beta$ -thalassemia. Among them five to six mutations account for about 80% of  $\beta$ -thalassemia mutations in Indian population (Jain V *et al.* 2006) [6]. Thus within each geographic population there are few common mutations together with a few rare ones which are responsible for over 90% of  $\beta$ -thalassemia mutations. The present study has been taken as there were no reports of mutations spectrum from Akola region.

**Correspondence:**  
**Dr.Z.H.Khan,**  
**HOD, Biochemistry**  
Shri Shivaji College of Arts,  
Commerce & Science, Akola  
(MS)

## 2. Materials and Methods

**2.1 Subjects:** The study sample consisted of 20 normal and 50  $\beta$ -thalassemia carrier subjects i.e. parents of known  $\beta$ -thalassemia major patients were included in the present study and their  $\beta$ -thalassemia carrier status was confirmed by red blood indices and study of hemoglobin variants separation by Cation exchange HPLC (Biorad) with HbA<sub>2</sub> level >3.5%.

### 2.2 DNA Analysis

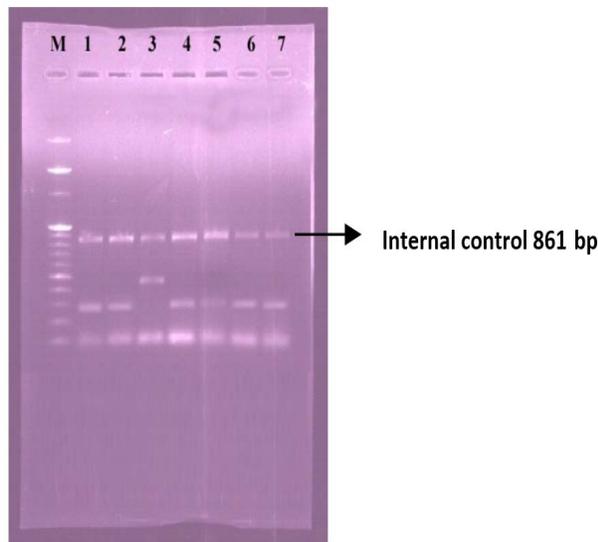
Blood samples were collected in EDTA tubes. DNA extraction was carried out by using DNA sure Blood Mini Kit NP 61105 of Genetix, according to manufacturer's protocol. The DNA yield was checked with 0.8% agarose gel electrophoresis. The extracted and purified DNA in 1.5 ml of micro centrifuge tube was then stored at 4 °C until further analysis.

Mutational analysis was carried out using the Semi-quantitative simple polymerase chain reaction (PCR). For this  $\beta$ -thalassemia detection kit of Himedia was used for studying 5 common mutation such as co 8/9(+G), co 41/42 (-CTT), IVS-I nt 5(G-C), IVS-1 nt1(G-T) and 619 deletion.

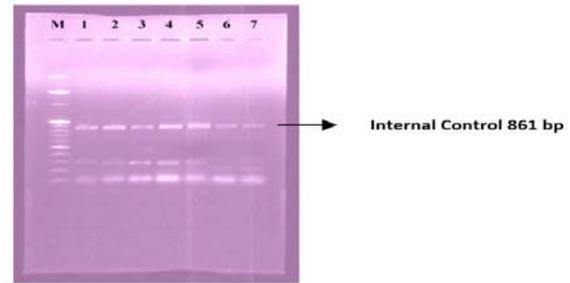
The PCR was carried out in a total volume of 25 $\mu$ l reaction mixture, containing 5 $\mu$ l of extracted DNA, 12.5 $\mu$ l of 2x PCR Master mix, 1 $\mu$ l of Primer mix, 1 $\mu$ l of internal control and 4 $\mu$ l of nuclease free water. Cycling was carried out on the thermal cycler with initial denaturation at 94°C for 5min 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.

After completion of PCR programme 10 $\mu$ l of PCR product was mixed with 3 $\mu$ l of gel loading dye and electrophoresed on 1.5% agarose gel containing ethidium bromide using TAE buffer for 1 hr at 100 volts. 100 bp DNA ladders were used for identification of band size. Gel was then visualized under Gel-doc system (Bioera)

## 3. Results



**Fig 1:** Showing Agarose gel electrophoresis of PCR. A product of 861 bp DNA band serves as internal control indicating proper reaction conditions. Lane M indicates 100 bp DNA ladders while Lane no. 1,2,4,5,6,7 showing the presence of mutation IVS-I nt.5(G-C) with band size 285 bp and Lane no. 3 indicating the mutation Co 41/42 (-CTT) with a band size 443 bp.



**Fig 2:** Showing Agarose gel electrophoresis of PCR. A product of 861 bp DNA band serves as internal control indicating proper reaction conditions. Lane M indicates 100 bp DNA ladders while Lane no. 1 & 5 showing the presence of mutation IVS-I nt.5 (G-C) with band size 285 bp and Lane no. 2,3, & 4 indicating the mutation IVS-I nt. 1(G-T) with a band size 281 bp, Lane no. 6 & 7 indicating the presence of Co 8/9 (+G) mutation having band size 214 bp.

**Table 1:** Mutational analysis of  $\beta$ -thalassemia carrier subjects

Mutation	No. of subjects	Percentage (%)
IVS I-nt 5 (G-C)	33	66
IVS I-nt 1 (G-T)	08	16
Codon 8/9(=G)	05	10
Codon 41/42 (-CTT)	01	02
619 bp deletion	00	00
uncharacterized	03	06
Total	50	

In the present study simple PCR based technique has been standardized for detection of common  $\beta$ -thalassemia mutations. Mutational analysis was performed on 50 subjects who were proven as carriers for  $\beta$ -thalassemia either during the screening or parents of known  $\beta$ -thalassemia major patients.

Blood samples of  $\beta$ -thalassemia carrier subjects were collected for DNA isolation and mutational analysis. Out of 50 subjects, IVS I-nt 5(G-C), a mutation of Asian-Indian origin was found to be the most common mutation. A total of 33(66%) subjects were reported to be affected with this mutation, a Mediterranean mutation, IVS I-nt 1(G-T) was found in 08 (16%) subjects, another Asian-Indian mutation Co 8/9 (+G) was found at low frequency in 05 (10%) subjects. There was only 01(2%) subjects who showed the Co 41/42 (-CTT) mutation, 619 bp deletion type of mutation was not recorded in the following study and there were only 03(6%) subjects who did not show any of the five mutations studied. In each of the normal DNA sample only 861 bp internal control was amplified after PCR and no  $\beta$ -thalassemia mutant bands were observed.

## 4. Discussion

$\beta$ -thalassemia is a group of heterogenous autosomal recessive disorder, where complete absence or reduced synthesis of  $\beta$ -globin chain occurs in haem protein of hemoglobin. Sometimes the excessive production of  $\alpha$ -globin chains leads to its deposition in RBC resulting in less or ineffective erythropoiesis. (Weatherall, 1994; Steinber *et al.* 2001) [18, 13]. In last two decades over 200 types of different mutations have been studied throughout the World. (Weatherall 1994) [18]

In our work, common mutations that are prevalent in the Indian subcontinent have been chosen for the study. Out of the six mutations studied, 619 bp deletion at the 3' end of  $\beta$ -globin gene makes it nonfunctional. Frame shift mutations codon 8/9 +1 (+G) and -4 codon 41/42 (-TCTT) change the ribosome reading frame and causes premature termination of

translation. Mutation IVS I-1(G–T) causes change in splice junction causing ineffective RNA processing. IVS I-5 (G–C) destroys consensus sequences around splice junction which are essential for splicing. (Haig HK Jr. *et al.*, 1988) [5]

IVS I-5 (G–C) substitution which is  $\beta^+$  mutation was found to be the most frequent mutation in our study. This is totally in agreement with previous studies, reported from India and the Indian sub-continent. In Maharashtra, incidence of IVS I-5 mutation was reported to be 54 % (Varawalla NY *et al.* 1991, Agrawal S *et al.* 2000) [14, 15, 1]. The incidence of this mutation reported at various regions from Indian sub-continent is, from Sindh region 12 %, Punjab 38 %, Gujarat 41 %, Bengal 60 %, and from Tamilnadu 81 %. The incidence reported from Tamilnadu is the highest reported incidence from any of the regions. Similar results were observed from Haryana (57 %), Uttar Pradesh (58 %), and eastern India (72 %) which correlate with our studies (Varawalla NY *et al.* 1991, Ambedkar SS *et al.* 2001, Panigrahi I & Marwaha RK, 2007) [14, 15, 3, 9].

IVS I-1 (G–T) is one of the common mutation among Asian Indians (Varawalla NY *et al.* 1991) [14, 15] and phenotype is of  $\beta^+$  thalassemia. It is second common mutation which is observed in our study in 08 (16 %) subjects. The frequency was found to be similar to the previous studies from different geographic regions in India, Haryana (10 %), Uttar Pradesh (11%), Eastern India (11 %), and southern India (5 %). In western India it was reported to be 20 % (Verma IC, *et al.*, 1997, Agrawal S. *et al.*1994 Vaz FE *et al.*2000) [17, 1, 16]. Satpute SB *et al.* (2012) [12] reported 9.52% of people affected with this mutation in their study.

Codon 8/9 (+G) mutation was present in 05 (10 %) subjects out of total 50 subjects studied. This mutation has been reported from northern India (11 %) and from Gujarat (8.6 %) (Bandyopadhaya A, *et al.* 1999, Agrawal S, *et al.* 1994, Madan N *et al.* 1998) [4, 1, 7]. Varawalla *et al.* reported incidence of this mutation from Sindh, Punjab, Gujarat, and Tamilnadu region.

Codon 41/42 (–TCTT) mutation is one of the common mutations found in the Indian subcontinent. In our study the mutation was found in only one subject (2 %) out of 50 subjects. Incidence of this mutation was observed throughout India, from north-west Pakistan to Bangladesh and Bengal, and from Punjab to Tamilnadu. The incidence of this mutation was found to be 20 % in Bengal, 10 % in Tamilnadu, in Maharashtra 7.2 %, in Haryana it was 9 %, 3 % in Uttar Pradesh, 6 % in Eastern India, 5 % in Punjab, and 2 % in western India (Varawalla NY *et al.* 1991, Panigrahi I & Marwaha RK, 2007) [14, 15, 9].

619 bp deletion was not found in our study. However spectrum of 619 bp deletion mutation in India was found to be 16% in Haryana, 14% in Punjab, 5% in Uttar Pradesh, 2% eastern India, 5% in Southern India and in Western India it was found to be 8% (Agrawal S. *et al.* 1994 Vaz FE *et al.*2000) [1, 16].

## 5. Conclusion

Identification of the exact mutation helps to define the severity of the phenotype, plan therapy, it was hope that these observations might form the basis for a comprehensive diagnostic database that would be useful not only for genetic counseling but for prenatal diagnosis also.

## 6. References

1. Agarwal S, Hattori Y, Agarwal SS. Rare beta thalassemia mutation in Asian Indians. *Am J Hematol.*

2000; 65(4):322-3.

2. Agarwal S, Naveed M, Gupta UR, Kishore P, Agarwal SS. Characterization of beta thalassemia mutation in 57 beta thalassemia families seen at Lucknow. *Indian J Med Res.* 1994; 100:106-10.
3. Ambekar SS, Phadke MA, Balpande DN, Mokashi GD, Khedkar VA, Bankar MP. The prevalence and heterogeneity of beta-thalassemia mutations in the western Maharashtra population: a hospital based study. *Int J Hum Genet.* 2001; 1(3):219-23.
4. Bandyopadhyay A, Bandyopadhyay S, Chowdhury MD, Dasgupta UB. Major b-globin gene mutations in eastern India and their associated haplotypes. *Hum Hered*1999; 49:232-5.
5. Haig HK Jr, Corinne OB. Molecular basis and prenatal diagnosis of beta-thalassemia. *Blood* 1988; 72(4):1107-16.
6. Jain V, Sachdev A, Lokeshwar MR. Prevention of thalassemia. In: Sachdeva A, Lokeshwar MR, Shah N, Agarwal BR editors *Hemoglobinopathies*. New Delhi: Jaypee Brothers Publishers, 2006, 94-104.
7. Madan N, Sharma S, Rusia U, Sen S, Sood SK. Beta thalassemia in northern India (Delhi). *Indian J Med Res.* 1998; 107:134-41.
8. Saleh-Gohari N, Bazrafshani MR. Distribution of  $\beta$ -Globin gene Mutations in Thalassemia minor population of Kerman Province, Iran. *Iranian J Health*, 2010, 69-76.
9. Panigrahi I, Marwaha RK. Mutational spectrum of thalassemia in India. *Indian J Hum Genet.* 2007; 13(1):36-7.
10. Q-H Mo, X-R Li, C-F Li, Y-L He, X-M Xu. A novel frameshift mutation (+G) at codons in a  $\beta^0$  thalassemia gene results in a significant reduction of  $\beta$ -globin mRNA values; *J Clin Pathol.* 2005, 923-926.
11. Harisha SA, Poojitha K, Devaki RN, Suma MN, Devananda D, Vinod kumar HR. Spectrum of Beta Thalassemia mutations detection in South Karnataka population by using ARMS-PCR. *International Journal of Scientific Research.* 2014, 31-33.
12. Satpute SB, Bankar MP, Momin AA. The prevalence of  $\beta$ -thalassemia mutations in South Western Maharashtra; *Indian J Clinical Biochemistry.* 2012, 389-393.
13. Steinber MH, Forget BG, Higgs DR, Nagel RL. *Disorders of hemoglobin*. New York: Cambridge University Press, 2001.
14. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of beta thalassemia mutation on the Indian subcontinent: the basis of prenatal diagnosis. *Brit J Haematol.* 1991; 78(2):242-7.
15. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of beta thalassemia mutation on the Indian subcontinent: the basis of prenatal diagnosis. *Brit J Haematol.* 1991; 78(2):242-7.
16. Vaz FE, Thakur CB, Banerjee MK, Gangal SG. Distribution of beta thalassemia mutation in the Indian population referred to diagnostic centre. *Hemoglobin* 2000; 24:181-94.
17. Verma IC, Saxena R, Thomas E, Jain PK. Regional distribution of thalassemia mutation in India. *Hum Genet* 1997; 100:109-13.
18. Weatherall SJG, Nienhuis AW, Majerus PH, Varmus H. *The Molecular Bases of Blood Diseases*. 2nd Edn. W. B. Saunders, Philadelphia, 1994, 157-205.