International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

STUDY OF MUTATIONS IN BETA GLOBIN GENE AT CODON 19 AND CODON 41/42 LOCUS IN THALASSEMIA PATIENTS

Anjali Kulkarni[1]*,
J.B.Chauhan[2], K.M.Singh[2], Krishna Naik,[2] Dhruti Kothari[2]

[1]Assist Professor,N V Patel College of Pure And Applied Sciences.
[2]Post Graduate Department of Genetics, Ashok and Rita Patel Institute of Integrated Study And Research in Biotechnology & Allied Science (ARIBAS),Vidyanagar, Anand -388121, Gujarat, India

ABSTRACT
Thalassemia is a heterogeneous group of genetically determined anemias due to an impaired synthesis of globin chains which are an integral part of the haemoglobin molecule. All normal haemoglobins are formed from 2 α-like chains and 2 non-α chain. Thalassemia affects 7% of the world's population. An estimated 3.9% of Indian population carries the thalassemia trait. Thalassemia is of two types (I) Alpha-thalassemia (II) Beta thalassemia. Beta thalasseamia is more common than alpha in Indian population. Beta globin gene is located on chromosome number 11. The present study was carried out to detect the mutation in codon19 and codon41/42 of beta globin gene. The detection technique includes isolation of DNA from peripheral blood of the thalassemia patients of Surat and Anand regions of Gujarat state. DNA was isolated by standard phenol: chloroform method. PCR-RFLP was used for detection of mutation in codon19 and codon 41/42 of beta globin gene with MaeII and TaqI restriction enzymes respectively. Analysis for mutation in codon19 of beta globin gene shows presence of mutation with frequency of 64.28%AA (mutants) and 17.86% AB (heterozygous) in thalassemic patients while absence of mutations in normal individuals . For Codon 41/42 it was found that mutation was absent in the thalassemic samples and normal individuals

Correspondence to Author

ANJALI KULKARNI

Assist Professor,N V Patel College of Pure And Applied Sciences

Email: manisha2878@yahoo.com

KEYWORDS : *Beta thalassemia, PCR-RFLP, Beta globin gene mutation, codon19 mutation, codon 41/42 mutation.*

INTRODUCTION

Thalassemia is a blood disorder in which the body makes an abnormal form of hemoglobin, the protein in red blood cells that carries oxygen. Thalassemia affects 7% of the world's population. An estimated 3.9% of Indian population or 1 in 25

carries the gene for thalassemia [1] .In India, around 80 to 90% of the 10,000 babies born each year with the disease die from it. In certain Indian communities like Assamese, Bengali, Gujarati, Maharashtrian, Marwari and Punjabi, as high as 25% population are silent carriers of defective thalassemia gene [1] . The thalassemia is classified according to which chain of the hemoglobin molecule is affected. In α thalassemia, production of α globin chain is affected, while in β thalassemia production of the β globin chain is affected. Thalassemia produces a deficiency of α or β globin, β globin chains are encoded by a single gene on chromosome 11; α globin chains are encoded by two closely linked genes on chromosome 16. Thus in a normal person with two copies of each chromosome, there are two loci encoding the β chain, and four loci encoding the α-chain. The disease thalassemia is controlled by an allele, which in homozygous condition produces the severe thalassemia, but in heterozygous condition results in a mild form of the disease. Inheritance pattern of beta thalassemia intermedia follows classical autosomal recessive pattern of inheritance. Thalassemia is a monogenic, autosomal recessive disease [2]. If large number of families are examined where both parents are carriers of beta thalassemia genes than 25% (1 in 4) of their offsprings will have homozygous beta thalassemia syndrome i.e. presenting as beta thalassemia major. 50% of the offsprings will be carrier of beta thalassemia gene and 25% of the offspring will be normal.

OBJECTIVE

The present study has been carried out to detect mutation in the beta-globin gene at codon 19 and codon 41/42 locus by Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique in thalassemia patients and normal individuals.

MATERIALS AND METHODS

The blood samples for the study were obtained from the 40 unrelated patients of thalassemia reporting to different hospitals and pathology laboratories from Vadodara, Surat districts of Gujarat and 40 Normal blood samples were obtained from students volunteer P.G. Department of Genetics, ARIBAS College, New V.V.Nagar with informed consent. Methods of collection and use of human samples were approved by the institutional ethics committee. The blood samples obtained were brought to college laboratory on ice for further use.

Genomic DNA was extracted by standard phenol/chloroform method [3] . The PCR reaction was carried out to amplify beta-globin gene at codon 19 and codon 41/42 locus by using primers reported by [4].

Polymerase chain reaction was performed using a Thermal Cycler. The PCR products were subsequently screened for RFLP with the restriction enzyme *MaeII* for codon 19 and *Taq I* for Codon *41/42*.

DNA was amplified using 10pmol/µl of each primer, 2X PCR master mix, DNAse free water 7.5 µl and DNA template 30ng/µl. This sample mix was subjected to thermocycler consisting of denaturation at 94^0C for 5 min, annealing at 59.2^0C for 45sec for codon19 and annealing at 55^0C for *Codon 41/42* 45sec and extension at 72^0C for 45sec and finally to 35 PCR cycles.

RESULTS

Amplified PCR products of *Codon 41/42* region of 312 bp fragment was screened for *Taq I* RFLP (plate 1) and 293bp fragment of Codon 19 was screened for *MaeII* RFLP.

Genotyping was done according to Pramoonjago[4]. In the present study for codon 19 three restriction patterns were observed in thalassemic patients i.e. genotypes AA, BB and AC and in normal individuals genotype AA and BB were observed (Plate 1). For *Codon 41/42* one restriction patterns was observed in Thalassemic patients i.e. genotypes AA, in normal individuals two genotype AA and AB were observed (Plate 2).

In the present study genotypes observed for codon 19 in thalassemic patients were 64.28%AA, 17.86% BB, 17.86% AB and in normal

individuals 7.41% AA and 92.59% BB. Here the most observed genotype in normal individuals is BB which indicates absence of mutation in normal individuals and in thalassemic patients most observed genotype is AA which indicates presences of codon 19 mutations. In codon19 of beta globin gene shows presence of mutation with frequency of 64.28%AA (mutants) and 17.86% AB (heterozygous) in thalassemic patients while absence of mutations in normal individuals.

For *Codon 41/42* in Thalassemic patients genotypes AA was observed. In normal individuals two genotype AA and AB were observed. In thalassemic patients are 100% AA and in normal patients 90% AA and 10% AB. Here the most observed genotype in normal well as in thalassemic patients is AA.

In the present study, it was found that *Codon 41/42* mutation was absent in the thalassemic samples studied.

DISCUSSION

The results of present study for codon 19, presence of mutation in Codon 19 locus of beta globin gene in accordance with Pramoonjago [4] i.e. presence of mutation in codon 19 while absence of mutations in normal individuals.

The results of present study is in contrast with Sinha [5] and Colah [6] i.e. presence of mutation in codon 19

The results of present study for codon 41/42 mutation i.e. less frequency of mutation at codon41/42 is in accordance with Pramoonjago [4]

The results of present study i.e. less frequency of mutation at codon41/42 in beta globin gene are in contrast with Thein [7] reported among the Beta-thalassemia group the frameshift mutations 41/42 at a frequency of 62%.

This difference in distribution could be a reflection of the difference in origin of the two groups of patients Beta-thalassemia .

Figures

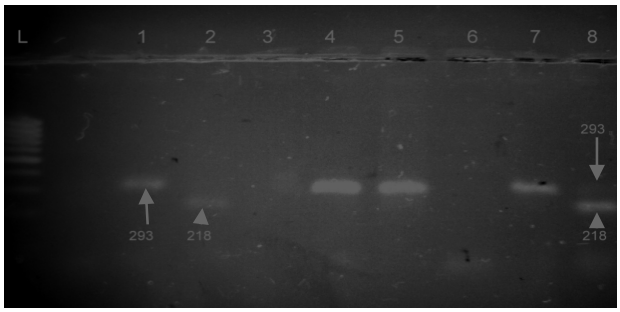

Plate 2:- Restriction digestion of codon 19 electrophoresed on 3% agarose
Lane 1, 4, 5, 7 – Mutant fragments of 293bp Lane 2 – Non mutant fragment of 218bp
Lane 8 – Heterozygous with 293bp and 218bp

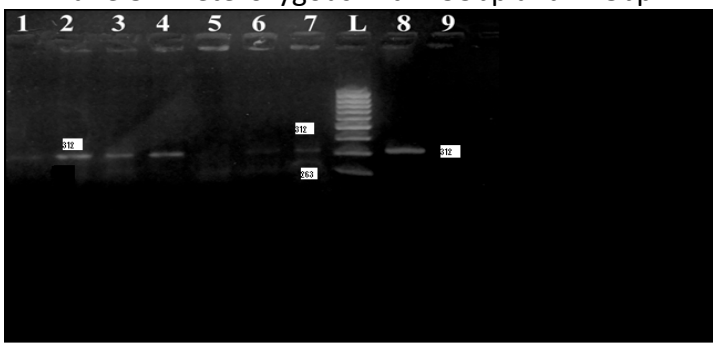

Plate 1: Restriction digestion of codon 41/42 electophoresed on 3% agarose
Lane 1,2,3,4,6,7- Restricted Product, Lane 8- PCR product , Lane L- 100bp DNA Ladder

REFERENCES

1. Ghodekar S. R., Grampurohit1 N. D, Kadam S. S. , Thorat R. M.(2010) Thalassemia: A Review International Journal of Pharma Research and Development – Online PRD/2010/ PUB/ ARTI / VOV-2/ISSUE-10/DEC /014
2. Deo, M.G., Gangal, S., Kher, A., 2006, Human Genetics, Moving Academy of Medicine and Biomedicine, topic 15, pp 15.1-15.14.
3. Sambrook, J. and Russell, D.W.(2001). Molecular cloning: A laboratory Manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY.
4. Pramoonjago, P., Harahap, H., Taufani, R. A., Setianingsih, I., Marzuk, S., 1999, "Rapid screening for the most common Beta thalassaemia mutations in south east Asia by PCR based restriction fragment length polymorphism analysis (PCR-RFLP)," J Med Genet; 36 pp: 937–938.
5. Sinha, S., Black, M. L., Agarwal, S., Colah, R., Das, R., Ryan, K., Bellgard, M., Bittles, A. H., 2009, "Profiling b-thalassaemia mutations in India at state and regional levels: implications for genetic education, screening and counselling programmes", Springer, HUGO J 3 pp 51–62.
6. Colah, R., Gorakshakar, A., Phanasgaonkar, S., D'Souza, E., Nadkarni, A., Surve R., Sawant, P., Master, D., Patel, R., Ghosh, K., and Mohanty D., (2009) "Epidemiology of b-thalassaemia in Western India: mapping the frequencies and mutations in sub-regions of Maharashtra and Gujarat," Blackwell Publishing Ltd, British Journal of Haematology, 149, pp 739–747.
7. Thein,S.,Hesketh,W.,S.Best,Fucharoen,S.,Wasia nd,D.and Weatherall,J.(1990).The Molecular Basis of beta thalassemia in Thailand:Application to Prenatal Diagnosis, Am. J. Hum, Genet,47,pp.369-375.

*****