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HBB gene exon-1 mutation analysis among caste population of Madhya Pradesh using Molecular genetic approach

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Abstract

Mutation in HBB gene may alter the production of beta globins. It is caused due to a number of variations like insertions or deletions in the HBB gene. The effects and types of mutations in the HBB gene can be studied by molecular identification followed by preparation of DNA using the FTA (Fast classic cards Technology),polymerase chain reaction (PCR) based technique and sequencing which is a potential molecular diagnostics technique which may lead to improved diagnosis, with earlier and more effective intervention strategies. The study is focused on analysis, screening and identification of HBB gene mutation with its different variants, in exon1 region of HBB gene on caste population of Madhya Pradesh using molecular genetic techniques as well as various bioinformatics tools for identification and confirmation of mutations.

Key Words: β-Globin Chains, HBB gene mutation, molecular genetics, Bioinformatics Tools.

1. Introduction

Hemoglobinopathies are a group of single gene disorder of primary structure of globin encountered universally. Several mutations associated with hemoglobinopathies have been documented in the β-globin gene cluster,the beta globin (*HBB*) is a gene which produced vital subunit of hemoglobin called beta globin or beta chain, the gene maps in short p-arm of chromosome number 11(18).Beta-thalassemia is a highly prevalent autosomal recessive disorder a type of hemoglobinopathie in which structural variation in β-globin gene is observed.It is characterized by the reduced or absent expression of the β-globin gene, most often due to the substitution of a single amino acid, resulting from abnormalities in the formation of the beta-globin moiety(17). Several hundred mutations have been reported that are produced in and around the globin gene, some of the variants exist at a polymorphic level in a number of populations while others are rare(13).The molecular polymorphism of the β-globin gene cluster has been extensively studied in human populations during the past 20 years. More than 300 different beta-globin gene mutations have been characterized.Most of the beta-thalassemia mutations are caused by point mutations, small deletions or insertions within the coding regions and the exon-intron junctions. The 5' gene region of the β-globin gene cluster that harbors fetal and embryonic genes exhibits high levels of linkage disequilibrium. A recombination hot spot was located within a 9-kb DNA segment separating the 5' sub haplotypes from the 3'part of the cluster (11), which includes the adult beta-globin gene. A polymorphic repetitive sequence located 500 bp upstream of the β-globin gene, in the vicinity of the recombination hot spot, is probably associated with the replication origin(8).The types of the mutation are typically ethnic specific(14,6).In the Mediterranean region, over ~50 β-thalassemia mutations have been characterized,in which IVS-I-110 (G→A)has high frequencies in the eastern part of the Mediterranean,

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while the mutation at codon 39 (C→T)is very frequent in western Mediterranean countries (4).Some *HBB* alleles with deletion mutations can be common in certain ethnic groups[e.g., the 619-bp deletion in Asian Indians, commonly found *HBB* mutations in Indian population are [COD 8/9(+G), COD 15(G-A),IVS 1:1(G-T),IVS 1:5(G-C), COD 30(G-C), IVS 1:1(GA), COD 41/42(-TCTT)&(COD 26(G-A)](13).Sequence analysis detects mutations in the *HBB* coding region and associated flanking regions. Deletion/duplication analysis detects variable extent of the *HBB* gene or of the beta-globin gene cluster that result in β-thalassemia or in the complex β-thalassemias.To make a clear diagnosis of individual status (9).Various DNA analysis techniques such as dot blot analysis, reverse dot blot, allele specific amplification using amplification refractory mutation system (ARMS) or direct DNA sequencing have been widely used to identify beta-globin gene mutations (2).In the present study, we report results of a study of the genetic diversity of the β-globin gene cluster in an ethnically well-defined population. The central Indian state Madhya Pradesh is often called as 'heart of India'. There are less detailed genetic studies on the populations inhabited in this region. Therefore, this study is an attempt for extensive characterization of mutation of two caste populations, inhabiting this region (19).Once the DNA sequence is known it would be easier to detect various types of mutations in the genome of an individual that give rise to different genetic and complex disease. Sequence analysis through Bioinformatics approaches shows the mutations in HBB gene. A large number of such genetic studies on the caste populations are required to bring as many as caste of the state on genetic map of India. Such type of study would be helpful in future not only in revealing as well as forming the foundation to understand the genetic basis of complex diseases found in caste population.

2. Materials and Method

2.1 Subjects

All the subjects and controls are native of MP, although the caste population i.e. Basod and Kunbi caste belonged to remote villages in districts of Madhya Pradesh. The process of sample collection was done in accordance with the ethical standards of Institutional Ethical Committee, following ICMR guidelines (IEC No: 1409-26/10/15).

For the present study about 2-5 ml of blood sample was collected from healthy as well as patients of the studying.Using 5ml disposable syringes in vials containing EDTA as anticoagulant and incidentally put away in to the refrigerator (4ºC) and immediately handled for further examination.

2.2 DNA extraction

Extraction of DNA samples was done using Whatman FTA classic cards. The methodology used is an advanced & more efficient technique for DNA isolation and PCR amplification .It is an alternate and advanced method of DNA isolation best suitable for population genetic study(7).

2.3 Amplification of exon 1 region

Amplification of β-globin gene exon 1 region was conducted with Polymerase Chain Reaction using forward primer5'CCAAGGACAGGTACGGCTGTCATC3',reverse primer5'CTATTGGTCTCCTAAACCTGTCTTG3'(10).

PCR mix was made with a total volume of 25mL, consisting of master mix (16 mL), 10 pmol forward and reverse primers (1µL each),DNA template 4 FTA discs and nuclease free water.The amplification conditions are as follows45 cycles with 2 holds initiation(95 °for 10 minutes,95 °c for 15sec,52 °cfor15sec,72°c for 15 sec. 72°c for 1 min,4°c infinity).

2.4Electrophoresis

Electrophoreses for the amplified products of exon 1 HBB gene was done at 120V in 0.8% agarose gel.The gel was visualized under ultraviolet light of UV trans-illuminator and the images are grabbed in the Gel Documentation system (BioEra) (12).

2.5*DNA Sequencing*

DNA sequencing was performed by using the Sanger (dideoxy nucleotide chain terminator) method with both forward and reverse primer has been done.Sequencing was done with the help of **Scigenome lab Pvt. Ltd.Cochin.**

2.6 Data analysis

Sequencing data were analyzed with software ChromasPro software (version .2.1.8).Sequences of HBB gene exon 1regions were sequenced with reference sequence of gene.The reference sequence for human beta-globin gene(*HBB*) >NG_000007.3(15),obtained from NCBI RefSeq database (http://www. ncbi.nlm.nih.gov/refseq/).

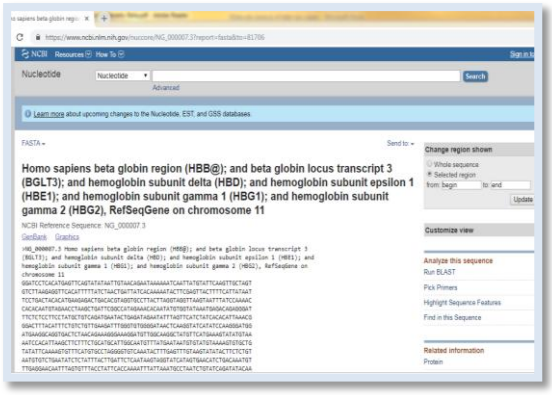

Figure.1-Refrence sequence retrived from NCBI

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Sequence Alignment: Multiple sequence alignment of nucleotide sequence of HBB gene as well as studied sequences of patients was carried out by the clustal W tool by using the MEGA6 software (version.06)(1,14).Further alignment was visualized with help of BioEdit V7.1.9 (1) software to observe the presence of deletions and synonymous mutations.

3. Results and Discussion

For the better amplification of DNA trapped on the FTA classic card, result of gradient PCR illustrated that the annealing temperature 52 °C for 15 sec was better for the *HBB* gene exon 1.On obtaining a single band devoid of any primer-dimer bands the PCR products were preceded for sequencing. Total 16 sequences of samples from caste population have been amplified with both forward and reverse primers of exon1 region of HBB gene.

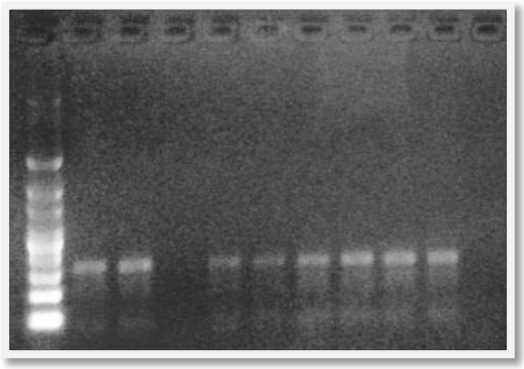

Figure2:Agrose gel image (2%)gene electrophoresis

Clean and sharp peaks without any noise shown in electropherogram confirmed that the PCR product is of good quality and the read length of DNA sequence is good and long which indicate that the DNA template is present in sufficient conc. and possible mutations can be analyzed by converting electropherogram in fasta formate using available bioinformatic tools.In the present study it is done by using ChromasPro softwar.

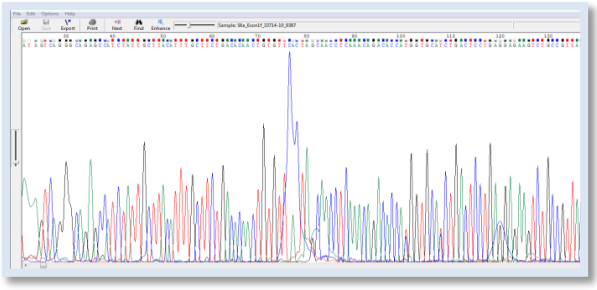

Figure.3-Electropherogram showing sequence of exon 1region of HBB gene

Total 16 sequences of *HBB* gene exon 1 were aligned with Reference Sequence HBB gene>NG_000007.3.The alignment was observed from nucleotide48 -378(Exon1 region). Terminal region is not considered for mutation due to poor quality of sequencing. Since the level of similarity depends on the species examined and can vary among different genes and even different regions within genes and genomic segments, Consequently mixed level similarities of conserved regions were observed among the studied samples of Kunbi, Basod castes population of Madhya Pradesh.Several mutations were encountered in the HBB gene exon 1 region of studied sequences .In the sequences insertion of A was observed in entire exon1 at position304. At position 76 deletion of A is observed in four subjects deleted and coding frame shift by one nucleotide. T >C type mutation was observed at position158, 170 and158 in most of the samples sequences. T>G type mutations at position 140.Low frequencies of A>C allelic form were observed, whereas high frequency of T>C type allelic form were observed equally distributed among all the studied subject sequences (11,9). The results of this study have helped to characterize the castes of Madhya Pradesh (M.P.) genetically.

1. Conclusions

The present study differs from studies for HBB gene mutation detection in respect of primer selected.The other studies focused on mutation screening by scanning full length HBB gene whereas the current study focused only on exon 1 region of the HBB gene.The type of mutation in HBB gene exon 1 in the represents nonsense mutations due to the deletion, insertion or inversion of a single nucleotide. Mutations can bring about a change in codon sequences, studies suggested that there is need to maintain a primary prevention program to analyze mutation, sequence variations at molecular level, it can help to overcome many genetic disorders. With some preliminary important actions and measures the identification of mutation in HBB gene will reduce health disparities in an already vulnerable population.

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