ORIGINAL RESEARCH PAPER

Biochemistry

SCREENING AND MOLECULAR CHARACTERIZATION OF HAEMOGLOBINOPATHIES USING ARMS (AMPLIFICATION REFRACTORY MUTATION SYSTEM) AND DIRECT DNA SEQUENCING TECHNIQUES AMONG YOUNG IN CENTRAL GUJARAT WESTERN INDIA

KEY WORDS: ARMS PCR, Central Gujarat, DNA sequencing, Haemoglobinopathies, Western India.

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ABSTRACT

Haemoglobinopathies is consider the most common inherited disorders in human and results from genetic mutation in one or more genes The present study was aimed to characterize the β-thalassemia mutation and haemoglobin variant in youth by ARMS PCR which is an uncomplicated and convenient method for identification of five common mutation from central Gujarat, western India region. This Study included 44 randomly selected haemoglobinopathies carrier student's sample of Anand People's Medicare Society (APMS), Anand for DNA analysis by ARMS PCR from March 2021-April 2021. Identification of five common Indian β thalassemia mutations along with Hb S and HB E were carried out by ARMS PCR method and δβ- thalassemia mutation was characterized by GAP-PCR. The samples which remain uncharacterized were sent to S N gene lab, Surat for DNA sequencing. In our study the most common mutation among five common mutations characterized was IVS-1, nt5 (G→C) in 22 (50%), followed by Codon41/42 (-CTTT) in 5 (11.3%). IVS-1, nt1 (G→T), Codon8/9 (+G) and 619bp del mutation was not identified in any carrier students screened for haemoglobinopathies. Other than these five common mutation Codon -88 (C→T) (2.27%) and Codon 30 (G→A) (2.27%) are also detected. The prevalence of haemoglobinopathies with respect to communities, reflects that SC/ST/OBC are at the highest risk with 50%. Communities like Rajput (22.7%), Patel (18.1%), Brahmin (6.8%) and Muslim (2.2%) are also showing prevalence. The study has included mutation in different communities reflects characterization of mutation of central Gujarat, western India which is significant for rapid and convenient identification of mutation while conducting screening programs and prenatal diagnosis. Rare mutations which are not recognized, need further confirmation for carrier detection followed by prenatal diagnosis.

INTRODUCTION

Haemoglobinopathies are group of diseases characterized by abnormalities both quantitative (thalassemia syndromes) and qualitative (sickle cell anemia) in the synthesis of haemoglobin[1]. Which is consider the most common inherited disorders in human and results from genetic mutation in one or more genes[2].

The curative treatment like bone marrow transplantation is costly, so prevention is the cost-effective strategy, which includes population screening, genetic counselling and prenatal diagnosis[3]. Amongst the various methods used for identification of mutation DNA analysis is most acceptable test because of its rapid diagnosis and safe termination of pregnancy is possible if required. There are 785 mutations have been identified on β-globin gene cluster, among them 232 mutations on β-globin gene are responsible to cause clinical phenotypes of β- thalassemia though only few specific mutations among these reported for each population. In India different region has disparate set of mutation and frequency of haemoglobinopathy[3]. Around twenty-two β-thalassemia mutations have been recorded in India, common mutation accounted for 80% are IVS I-5 (G-C), IVS I-I (G-T), Codon 41/42 (-TCTT), Codon 8/9 (+G) and 619bp deletion[4].

In countries with high prevalence of hemoglobinopathies, premarital screening programs should aim to identify asymptomatic carriers of haemoglobin disorders in young population to assess the risk of having children with severe form of disease.

MATERIAL & METHODS:

This Study included total 44 randomly selected haemoglobin opathies carrier sample of students (age group 18 to 30 years) of Anand People's Medicare Society (APMS), Anand for DNA analysis by ARMS PCR from March 2021-April 2021 at Indian red cross society, Gujarat State branch, Ahmedabad. Samples characterized for common mutation are IVS I-5 (G-C), IVS I-I (G-T), Codon 41/42 (-TCTT), Codon 8/9 (+G) and 619bp deletionby ARMS-PCR.

A 2-ml intravenous blood sample was collected in EDTA according to practical manual of Dacie[5]. A stepwise identification approach has been applied to characterize the mutation. This involved the following[6,7]:

A. DNA isolation from blood with commercially available Qiamp DNA Mini Kit & Maxwell® RSC Whole Blood DNA Kit from the peripheral venous blood of the subjects having BTT, SCT and other hemoglobin variants. QIAxpert is a high-speed microfluidic UV/VIS Spectrophotometer used to determine specific amounts of DNA and profiles sample content to differentiate between DNA, RNA and sample impurities.
B. Detection of the five common Indian β thalassemia mutations along with Hb S and Hb E by ARMS PCR.
C. Detection of mutation in δβ -thalassemia and HPFH by GAP-PCR.
D. The samples which remain uncharacterized are sent to SN gene lab, Surat for DNA sequencing.

Ethical considerations:

The Ethics board of H M Patel Center for Medical care & education, Karamsad, faculty of medicine approved this study. Written informed consent was obtained from all students involved in the study.

RESULTS:

From 44 randomly selected carrier students were analyzed for mutation of different haemoglobinopathies. For mutation characterization ARMS-PCR method was used to identify five common β- thalassemia mutation and Hb S as well as Hb E mutation. Uncharacterized mutations were sent to SN gene lab for DNA sequencing. Among them 27 (61.3%) students were

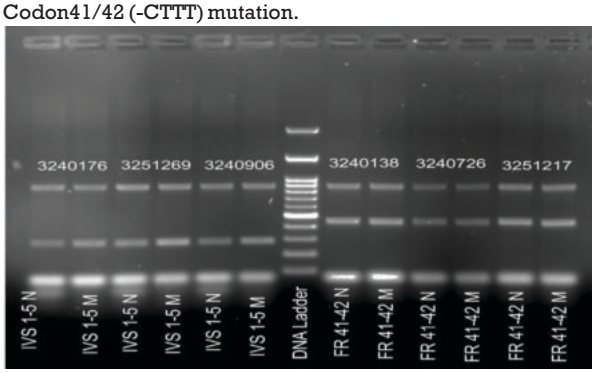
characterized by five common mutation, 2 (4.5%) students were found to have rare mutation. 10 (22.7%) students having Hb S Codon 6 (A→T) heterozygous mutation and 1 (2.27%) student was identified for Hb S Codon 6 (A→T) Double Heterozygous mutation. 2 (4.5%) students were identified Hb E Codon 26 (G →A), 1 (2.27%) student found to have Asian Indian Inversion (Aγδβ)° δβ-thalassemia and 1 (2.27%) mutation was not detected by DNA sequencing.

The most common mutation among five common mutations characterized was IVS-1, nt5 (G→C) in 22 (50%), followed by Codon41/42 (-CTTT) in 5 (11.3%). IVS-1, nt1 (G→T), Codon8/9 (+G) and 619bp del mutation was not identified in any carrier students screened for haemoglobinopathies. Other than these five common mutation Codon -88 (C→T) (2.27%) and Codon 30 (G→A) (2.27%) are also detected. The frequency of these mutation is shown in the table I.

Table I: shows distribution of various mutations in different hemoglobinopathies.

Types of mutation	Number of case positive	Mutation Frequency (%)
Common (n= 40) by ARMS-PCR		
Hb S Codon 6 (A→T) Heterozygous	10	22.72%
Hb S Codon 6 (A→T) Double Heterozygous	1	2.27%
IVS-1, nt5 (G→C)	22	50%
Codon41/42 (-CTTT)	5	11.36%
IVS-1, nt1 (G→T)	0	0
Codon8/9 (+G)	0	0
619bp del	0	0
Hb E: Codon 26 (G→A)	2	4.54%
Rare (n= 04) by DNA sequencing		
Asian Indian Inversion (Aγδβ)°	1	2.27%
Codon -88 (C→T)	1	2.27%
Codon 30 (G→A)	1	2.27%
Unidentified mutation	1	2.27%
Total	**44**	**100**

Fig: Agarose gel of ARMS-PCR showing IVS-1, nt5 (G□C) and Codon41/42 (-CTTT) mutation.



The prevalence of haemoglobinopathies with respect to communities, reflects that SC/ST/OBC are at the highest risk with 50%. Communities like Rajput (22.7%), Patel (18.1%), Brahmin (6.8%) and Muslim (2.2%) are also showing prevalence.The distribution of different mutation with respect to communities is shown in table II. This data indicates IVS-1, nt5 (G→C) is most common mutation seen in all communities except Muslim. Second common mutation is Codon 41/42 (-CTTT) found in Rajput, Brahmin and SC/ST/OBC caste. Hb S Codon6 (A→T) Heterozygous and doble heterozygous mutation is more prevalent in SC/ST/OBC caste. Where Hb E Codon 26 (G→A) is found in Muslim as well SC/ST/OBC caste. δβ-thalassemia Asian Indian Inversion (Aγδβ)° is found in Rajput caste and rare mutation Codon -88 (C→T) is identified in Patel community and Codon 30 (G →A) is characterized in Rajput community. One unidentified mutation belongs to SC/ST/OBC caste.

Table II: shows frequency distribution of mutations with respect to community.

Community	IVS-1, nt5 (G→C)	Codon 41/42 (-CTTT)	Hb S Codon6 (A→T) Hetero zygous	Hb E: Codon 26 (G→A)	Asian Indian Inversion (Aγδβ)°	Codon -88 (C→T)	Codon 30 (G→A)	Unidentified mutation	Total (%)
SC/ST/OBC	10	01	09	01	00	00	00	01	**22(50%)**
Rajput	05	02	01	00	01	00	01	00	**10(22.7%)**
Patel	06	00	01	00	00	01	00	00	**08(18.1%)**
Brahmin	01	02	00	00	00	00	00	00	**03 (6.8%)**
Muslim	00	00	00	01	00	00	00	00	**01(2.27%)**
Total	**22**	**05**	**11**	**02**	**01**	**01**	**01**	**01**	**44 (100%)**

DISCUSSION:

From randomly selected 44 carrier students two common mutations were characterized in 61.3% (Table IV). among the haemoglobinopathies mutation, IVS1-5 (G→C) and Hb S Heterozygous are the most common mutation, occurring at 50% and 25%, respectively. Frequency of IVS1-5 (G→C) mutation is found high in many other parts of India including Gujarat, Maharashtra, Punjab and Southern India[8,9]. In Eastern region of India frequency of IVS1-5 (G→C) was reported 72% [10]. In another study it was reported that the prevalence of IVS1-5 (G→C) ranges from 44.8% in North to 71.4% in East region of India and to western region the prevalence of IVS1-5 (G→C) mutation was recorded to be 54.7%[11]. the present study in concordance, the frequency of IVS1-5 (G→C) mutation is similar (50%) totally in agreement with previous studies.

The study has revealed the frequency of Codon41/42 (-CTTT) mutation is comparatively high 11.3%. In India the frequency of this mutation was observed 20% in Bengal, 10% in Tamandu, 9% in Haryana, 7.2 % in Maharashtra, 6% in eastern India, 5% in Punjab, 3% in Uttar Pradesh and 2% in Western India[12,13].

The present study has revealed the absence of IVS-1, nt1 (G□T), Codon8/9 (+G) and 619bp del mutation from carrier students screened for haemoglobinopathies. Previous studies have been shown high prevalence of these mutations from Gujarat[14,15].

On the bases of community distributions shown in table II, it depicts that SC/ST/OBC communities are at high risk, with IVS1-5 (G→C) mutation 50%, followed by Hb S Codon6 (A□T) Heterozygous and doble heterozygous mutation 25%, Hb E Codon 26 (G→A) mutation and Codon 41/42 (-CTTT) mutation 2.27%. Whereas Rajput (22.7%), Patel (18.1%), Brahmin (6.8%) and Muslim (2.2%) communities followed sequentially, being most affected by IVS1-5 (G→C) mutation. The present study reflects mutation among native Indians[14].

Second common mutation is Codon 41/42 (-CTTT) which shares its prevalence in Rajput (4.5%), Brahmin (4.5%) and SC/ST/OBC (2.27%) caste. It shows similarity with previously publish data[14].

The frameshift mutation Codon -88 (C□T) and Codon 30 (G →A) are considered as rare mutations in Indian population but are characterized with low frequency (2.27%) in Rajput and Patel community. Occurrence of rare mutation in certain communities has eased to more specific screening.

In present study Hb E Codon 26 (G→A) mutation is found in Muslim as well SC/ST/OBC caste and δβ-thalassemia Asian Indian Inversion (Aγδβ)° is found in Rajput caste. One unidentified mutation belongs to SC/ST/OBC caste (Table V).

Characterization of mutation pattern reveals from such study provides the basis for prenatal diagnosis and genetic counselling of affected individuals. It is necessary to find out reason for exceptional phenotypic heterogeneity and inherent history of these disorders to conserve rare resources the most cost-effective methods should be established for their control and management[16].

The study has included mutation in different communities reflects characterization of mutation of central Gujarat, western India which is significant for rapid and convenient identification of mutation while conducting screening programs and prenatal diagnosis. Rare mutations which are not recognized, need further confirmation for carrier detection followed by prenatal diagnosis.

Conflict of Interest: None

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