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Identification of Gene Mutation in Sickle Cell Anemia in Tribal Population of Rajasthan

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ABSTRACT: Sickle cell disease (SCD) is a genetic disorder caused due to change in single amino acid substitution (β6Glu>Val) that causes sickling of the red blood cells. SCD has different genotypes with substantial variations in presentation and clinical course. Total 07 samples (03 HbSS, 03 HbAS and 01 Normal) were sent to Imperial Life Sciences, Gurgaon to identify different mutation. Blood sample were collected in camps organized in schools and community. The blood sample was collected in EDTA vials to prevent clotting and preserved in 20°C for future analysis. The AGENA Mass ARRAY platform technique was used to detect SNPs, somatic mutations and in-dels using MALDI-TOF based detection. Out of 07 samples, In 03 samples we found two variant alleles (Homozygous), Hb S were detected in the given blood sample. Since, samples have two homozygous variants; there was complete inactivation of the beta globin chain. Thus, the disease indication was Sickle Cell Anemia. In other 03 samples we found One normal and one variant allele (Heterozygous), Hb S were detected in the given blood samples. Since, these samples have one normal and one variant allele; there is incomplete production of the beta globin chain. Thus, making this a carrier and the disease indication is Sickle Cell trait. In 01 sample we didn't found any mutation. Based on the result of the mutation identified and can thus be used for the future work in this area. In future we can do more mutation study and work can be extended with wide range of samples. Genetic counseling is recommended. Family members are recommended for screening in order to know the inheritance pattern and risk of disease occurrence in future generations.

Keywords: Sickle cell Anemia, tribal, Mutation, AGENA Mass ARRAY, MALDI-TOF

Abbreviations: SNP, Single-Nucleotide Polymorphism; MALDI-TOF, Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight.

I. INTRODUCTION

Sickle cell Anemia (SCA) along with Sickle cell Disease(SCD), the most common genetic disorders worldwide, characterized by sickling of red blood cells, due to vaso- occlusion and anemia [1]. Sickle cell disease may lead to various acute and chronic complications like; Acute painful episode, Anemia, Sequestration crisis, Acute Chest syndrome, Renal Complication, Priaprism, Dermetological complication etc. Presently, Born marrow transplant is the only therapy which is being used but it is costly and unaffordable for the general population, while hydroxyurea drug has also been used by the practitioner to reduce pain crisis and also used IFA tablets to increase Hb level in the patients. Approximately 312,000 people are born with HbSS worldwide [2]. The sickle cell Anemia commonly found in ethnic group of worldwide. In Sub-Saharan Africa the highest 236,000 people born with HbSS [3]. In India also the several ethnic group of several region reported the presence of Sickle Cell Anemia among different ethnic group from 1-40 percent [4]. In Rajasthan also we have 12 different tribes and 1 primitive tribe viz Bhil, Meena, Garasiya, Kathodi, Pargietc [5]. According to the census 2011, Rajasthan has 90 lakh tribal population [6]. Several studies have also been conducted on prevalence and phenotype of sickle cell Disease in Rajasthan, reported with 8.53 percent prevalence [7]. In SCA valine replaces Glutamic acid at 6 position of the β globin chain [8]. Several studies carried out on gene mutation in SCD worldwide showed that SCD variant is the result of GAG→GTG nucleotide change [9-10]. In our study we also tried to identify the presence of mutation in SCA subjects. Increased awareness of SCD and regular screening and monitoring may reduce disease-related morbidity and mortality in these populations. Genetic counseling for SCD has been shown huge acceptance in some parts of tribal areas of India; success rate of up to 50%, after a 5-year follow-up period [11].

II. MATERIAL AND METHODS

A. Selection of Sample size

A cross sectional study was carried out to identify different haemoglobin variants and their clinical manifestation on inhabitants of different tribal group of the Abu Road block of Sirohi district of Rajasthan. A stratified random sampling was used to conduct the study. We divided our study area in different strata like block, schools, classes and in the age group. Total 466 subjects were recruited on the basis of the purposive random sampling between the age group of 6-21 years to find out the different haemoglobin variants in the areas and also to develop clinical association with haemoglobin variants. Out of 466 subjects 234 subjects were reported HbAA and remaining 232 reported HbSS and HBAS.

B. Study Area

The study was carried out in different schools, maabadi of Abu Road block of the Sirohi district of Rajasthan for the proposed study. As the problem of sickle cell is more prevalent in an ethnic group thus we selected the southern district of Rajasthan which have concentration of the tribal population and already reported prevalence of the disease in the area.

C. Materials

Detection of the SCD in the field. All subjects were screened in the field by using solubility test conducted by the investigator. Based on the result of the solubility test we further collected 2 ml venous blood from all negative as well as positive subjects for further examination of the Hb variants through HPLC Bio-Rad Variant II. All samples were transported to Laboratory of the National Institute of Implementation Research on Non Communicable Disease (ICMR), Jodhpur to examine the haemoglobin variants in the samples. Out of the total samples, 07 samples (03 HbSS 03 HbAS and 01 Normal) were sent to Imperial Life Sciences, Gurgaon to identify different mutation.

DNA Extraction. As per the study protocol we have to identify the gene mutation in subjects of SCA. Total 07 sample, 06 positive and 01 control subjects were selected for identification of gene mutation. 5 ml venous blood was collected in EDTA tube. All the samples were sent to Imperial science, Gurgaon for further process of Genomic DNA. The samples were extracted from 200uL of blood using a Chemagic DNA blood extraction. All of the samples were send to the imperial lifescience Gurgoan, maintaining the proper cold chain.

Mass Spectrometry Assay. After DNA extraction the samples mass spectrometry assay was conducted which was designed to detect and identify gene mutation from 20 common Indian mutation. The MALDI-TOF (MA4-Agena Biosciences, San Diego, CA) was used for the analysis. DNA was amplified by PCR. The shrimp alkaline phosphatase was used to remove residual nucleotides. We outsourced the process of identification of gene mutation from Imperial sciences.

D. Statistical Analysis

Microsoft excel 2000 was used to calculate Call Rate and Extension Rate. Peak areas were calculated by TYPER software and transferred to Minitab for analysis.

III. RESULT & DISCUSSION

Table 1 represents the common gene mutation found in the Indian community. Total 20 mutations are commonly known in Indian community which has given below in Table 1. We also processed our samples to find out the mutation which present in our study area so we could develop correlation between HbS variant with clinical complication like acute chest pain, enlargement of spleen, bodyache and Anemia which is also a common problem found in young children and pregnant women. (12). Total 7 samples have been analyzed for the identification of mutation out of which the HbS were detected in our study area. However, it has already been proven that in different geographical and different community the different variant are present. HbS is the common problem found in the southern part of the Rajasthan. Thus we found only HbS mutation in the study subjects. In 03 samples we found two variant alleles (Homozygous), Hb S were detected in the given blood sample. Since, samples have two homozygous variants; there was complete inactivation of the beta globin chain. Table 2 depicted identification of mutation, HGVS (HBB: c. 20A>T), genotype and clinical significance of the subjects. In all six samples mutation found, results in the substitution of adenine to thymine at the 6th position of the hemoglobin beta gene. In first three sample (sample 1,2,3) two abnormal allele of the hemoglobin beta gene were found i.e homozygous (βHbS/βHbS)- Sickle Cell Anemia, whereas in sample 4,5,6 one abnormal allele of the hemoglobin beta gene were found *i.e* heterozygous (β/βHbS)- Sickle Cell Trait. We observed that clinical complications were more commonly found in homozygous state due to change in both allele as compared to heterozygous state in which only one abnormal allele was found.

Table 1: Identification of Gene Mutation in Sickle Cell Anemia in Tribal Population.

S. No.	Mutation	Sample no.1	Sample no. 2	Sample no. 3	Sample no. 4	Sample no. 5	Sample no. 6	Sample no. 7
1	IVSI-5(G>C)	-	-	-	-	-	-	-
2	619bpDEL	-	-	-	-	-	-	-
3	IVSI-1(G-T)and (G-A)	-	-	-	-	-	-	-
4	COD 41/42	-	-	-	-	-	-	-
5	COD8/9(+G)	-	-	-	-	-	-	-
6	COD15(G-A)	-	-	-	-	-	-	-
7	COD30(G-C) &	-	-	-	-	-	-	-
8	CAPSite+1(A-C)	-	-	-	-	-	-	-
9	COD5(-CT)	-	-	-	-	-	-	-
10	COD16(-C)	-	-	-	-	-	-	-
11	-88(C-T)	-	-	-	-	-	-	-
12	HbE	-	-	-	-	-	-	-
13	HbS	**Homozygous**	**Homozygous**	**Homozygous**	**Heterozygous**	**Heterozygous**	**Heterozygous**	No Mutation
14	HbD-Punjab	-	-	-	-	-	-	-
15	PolyASite	-	-	-	-	-	-	-
16	IVSII-837(T>G)	-	-	-	-	-	-	-
17	-28(G-A)	-	-	-	-	-	-	-
18	-90(C-T)	-	-	-	-	-	-	-
19	COD15(-T)	-	-	-	-	-	-	-
20	IVSI25-bp	-	-	-	-	-	-	-

Table 2: Detection of Different Haemoglobin Variants in Tribal Population.

Sample no.	Variant Identified	HGVS Name	Genotype	Clinical significance
Sample 1	Hb S	HBB: c. 20A>T	Homozygous (β^{HbS}/β^{HbS})	β^{HbS}/β^{HbS} Sickle Cell Anemia
Sample 2	Hb S	HBB: c. 20A>T	Homozygous (β^{HbS}/β^{HbS})	β^{HbS}/β^{HbS} Sickle Cell Anemia
Sample 3	Hb S	HBB: c. 20A>T	Homozygous (β^{HbS}/β^{HbS})	β^{HbS}/β^{HbS} Sickle Cell Anemia
Sample 4	Hb S	HBB: c. 20A>T	Heterozygous (β/β^{HbS})	Sickle Cell trait
Sample 5	Hb S	HBB: c. 20A>T	Heterozygous (β/β^{HbS})	Sickle Cell trait
Sample 6	Hb S	HBB: c. 20A>T	Heterozygous (β/β^{HbS})	Sickle Cell trait
Sample 7	-	-	-	Normal Hemoglobin

Similarly in another study carried out in Saudi Arabia also reported same mutation i.e. mutation in A→T in 6 person while they also reported mutation from A→C,G which has not been found in our study area [13]. Thus, making this a carrier and the disease indication is Sickle Cell trait. In control sample we didn't found any mutation, sample was HbAA (Normal).

IV. CONCLUSION

We found HBB: c. 20 A→T mutation in our study which has already been reported by different studies [14]. Clinical correlation is suggested and further genetic counseling is recommended. The limited studies have been carried out in the different ethnic group in different areas. Therefore it is very difficult to identify the gene prevalent in different areas. Therefore we may state that more studies need to carry out on the present problem.

V. FUTURE SCOPE

As it is a genetic disorder and caused by the genetic mutation the Genetic counseling along with marriage and prenatal counseling to the community to prevent further spread of the disease. Family members are recommended for screening in order to know the inheritance pattern and risk of disease occurrence in future generations.

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Conflict of Interest: NIL

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