

RFLP ANALYSIS OF SICKLE CELL DISEASE IN RURAL AND URBAN POPULATION OF CHHATTISGARH STATE

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ABSTRACT

Sickle cell disease is a major genetic disorder amongst scheduled caste, scheduled tribe and other backward communities population of chhattisgarh. For prevention programs involving population screening with the option of prenatal diagnosis for carrier couple. Here we find out the prevalence of sickle cell anemia in different community of chhattisgarh by using RFLP analysis with Dde 1 restriction enzyme. Here we report the β -globin gene analysis found in 150 randomly selected sickle cell patients, including 100 sickle cell anemia (SS), 30 Sickle cell trait (AS) and 20 normal individual. SCA were more frequently observed in Teli (39%), Kurmi (30%) and Rawat (9%) community. And in the case of SS fragment sizes generated by Dde 1 are significantly larger than those generated by AS. Normal individual had a 201 bp and 175 bp fragment after restriction digestion and Sickle cell trait show three band 201bp, 175bp and 376bp. sickle cell patients show single band 376 bp with the loss of 175 bp fragment. We interpret these change in banding pattern to be the result of the elimination of a restriction site for Dde 1 in the altered codon associated with sickle cell allele. Because an analysis can be performed on little amount of DNA, the application to prenatal diagnosis of sickle cell anemia should be possible.

KEYWORDS: Sickle Cell Anemia, β -globin gene, Hb Variants, PCR-RFLP, HbS, prevalence.

INTRODUCTION

Sickle cell disease results from homozygosity of the sickle cell allele (β^S) at the β -globin gene locus. The β^S allele differs from the wild-type allele (β^A) by substitution of an A (Adenine) in the wild-type to a T (Thymine) at the second position of the sixth codon of the β -chain gene, resulting in the replacement of a glutamic acid by a valine in the expressed protein due to which in deoxygenated state, shape of erythrocytes change to sickle shape and also the fragility of cell membrane increases.

The highest prevalence of Hb S is in tropical Africa and among blacks in countries that participated in the slave trade. It occurs with lower frequency in the Mediterranean basin, Saudi Arabia, and parts of India. India caters to nearly 20 million people with sickle cell disease.^[1] The sickle cell gene in India was first described among tribal groups in south India,^[2] which spread the wrong message that the disease is confined to tribal people. But the recent data unfolds that disease is not restricted only to tribal belt but widely prevalent and has penetrated different castes and communities in our country.^[3,4] It is widely recognized especially in the central parts of India.^[5] The highest frequency of sickle cell gene in India is reported in Orissa followed by Assam, MP, UP, Tamilnadu and Gujarat.^[6,7]

First diagnosis of sickle cell anemia affected individuals based on the linkage analysis of the sickle cell allele to the *Hpa* I restriction fragment length polymorphism.^[8] Later, it was shown that the mutation itself affected the cleavage site of both *Dde* I and *Mst* II and could be detected directly by restriction enzyme cleavage.^[3,4,5]

At present no past history of sickle cell anemia in Chhattisgarh is available and molecular study is also not done, therefore genetic associations studies, which attempt to link gene polymorphisms with selected disease sub phenotypes may eventually provide useful methods of foretelling the likelihood of certain complications and allow better individualized treatment.

The main objective of the present study is by using restriction fragment length polymorphism (RFLP) direct analysis of sickle cell anemia was done. This restriction endonuclease recognizes the DNA base sequence C-T-N-A-G, which is present in normal DNA at codon 5 and 6 but not in the β^S allele due to the single point mutation. The general impression in chhattisgarh is that, this disease is one of the major health problems. Therefore a community based study was taken up to assess the problem in this regards within its limited scope and resources.

MATERIALS AND METHODS

Patients with sickle cell anemia (SS), sickle cell trait (AS) and control individuals with no known hematological disorder (AA) were the subjects of this study. Subjects were selected from rural and urban region of Chhattisgarh, India. 5 ml peripheral blood sample was collected in EDTA coated vial. The population was screened by dithionite tube turbidity test (DTT) or solubility test and positive samples were subjected for Cellulose Acetate Membrane electrophoresis. DNA from peripheral blood sample was isolated by phenol/chloroform extraction method and then collected by ethanol precipitation. Concentration of DNA was checked in 1 % Agarose gel (Figure1) Specific β -globin gene (444-bp size) was amplified by PCR (Figure 2). The sequence of Forward and Reverse Primer was: Forward- 5'-ACCTCACCTGTGGAGCCAC-3' Reverse- 5'-GAGTGGACAGATCCAAAGGACTCAAAGA-3'. Amplified product was analyzed by 1 % Agarose gel electrophoresis then it was extracted from gel (Invitrogen). Amplified gel extracted product (Figure 3) was subjected to restriction digestion with *Dde* I (5units/ μ l) restriction enzyme for the analysis of variation in restriction endonuclease in the β -globin sequences (Figure 4 & 5).

RESULTS

The mean age of patients suffering from sickle cell anemia was 20 years. The hematological data showed the expected degree of anemia and hemolysis. The primary detection of sickle hemoglobin was done by dithionite tube turbidity test (DTT or solubility test) and positive samples were subjected for Cellulose Acetate Membrane electrophoresis.

In this study, polymorphism in the restriction endonuclease digestion pattern of a human DNA fragment that contains the β -globin structural gene was detected. Individuals with normal allele shows two bands of 175-bp and 201-bp. Sick cell trait individuals exhibit an additional band of 376-bp. Individuals with sickle allele show a single band of 376-bp due to the elimination of restriction site for *Dde* I (Figure 4 & 5). Total 151 samples were collected for the study. Among them, 100 individuals were found to be sickle cell diseased, 30 individuals found to be sickle cell trait and the rest 20 individuals were found to be normal. On the basis RFLP analysis prevalence of sickle cell disorder and sickle cell trait in different community shown in (Table 1). Kurmi (30%), Teli (39%) Rawat (9%), Gada (3%), Agharia (4%), Satnami (3%), Lohar (2%) and Bengali-halder (3%) castes) these population having single band after digestion. Prevalence of sickle cell disorder is higher in Teli (39%), Kurmi (30%) and Rawat (9%) caste.



Figure 1: Gel picture showing the isolated genomic DNA (>10 Kb) Lane 2-12: isolated genomic DNA from peripheral blood, Lane 1:100 bp DNA ladder, Lane 13: 1kb DNA ladder (range-1 kb-10 kb).



Figure 2: Gel picture showing the PCR amplified product (444 bp) of specific β -globin gene. Lane 1-6: PCR amplified different DNA sample of sickle cell anemia, Lane 7: 100 bp DNA ladder.

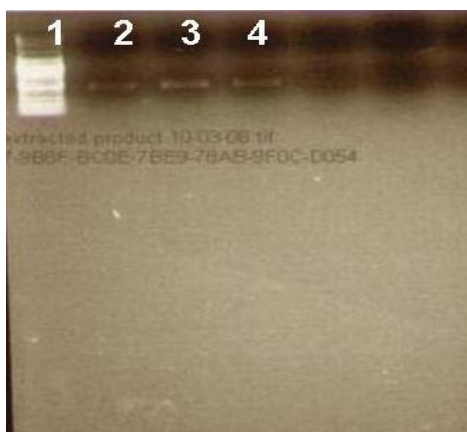


Figure 3: Gel extracted product of PCR amplified DNA (444bp) Lane 1: Gel extracted PCR product, Lane 2: 100 bp DNA ladder.



Figure 4: Gel picture showing Dde-I digested PCR product. Lane1-100bp DNA ladder, Lane 2: normal individuals (AA) showing 175 and 201 digested product. Lane 3-4: sickle cell trait sample (AS) of 175, 201 and 376 product. lane 5: 1 kb ladder.

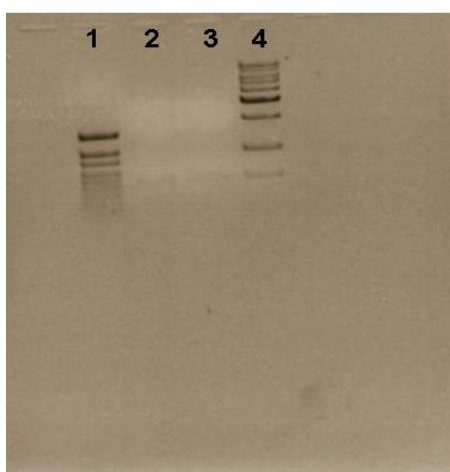


Figure 5: Gel picture showing Dde-I digested PCR product Sickle cell anemia patients. Lane 1 and 4: 100bp and 1 kb ladder. Lane 2-4 –Sickle cell anemia (SS) undigested product.

Table 1: Prevalence of sickle cell disorder as per community population of Chhattisgarh state.

Caste	Male	Female	Prevalence	% of prevalence	
				Male	Female
Teli	17	22	39	43.5	56.4
Kurmi	10	20	30	33.3	66.6
Rawat	3	6	9	33.3	66.6
Lohar	0	2	2	-	100
Gada	3	0	3	100	-
Satnami	1	2	3	33.3	66.6
Gond	0	1	1	-	100
Aghariya	1	3	4	25	75
Thakur	5	1	6	83.3	16.6
Bengali -(Halder)	1	2	3	33.3	66.6

Table 2: Prevalence of sickle cell trait as per community population of Chhattisgarh state.

Caste	Male	Female	Prevalence	% of Prevalence	
				Male	Female
Teli	5	7	12	41.6	58.3
Kurmi	4	5	9	44.4	55.5
Lohar	3	2	5	60	40
Rawat	3	0	3	100	-
Yadav	2	0	2	100	-
Total	17	14	31		

DISCUSSION

In the present study, solubility (DTT) test was used for screening test. Bankar *et al*⁹ and Kate⁴ have also recommended the solubility test as a screening test. In the present study, of *Dde*-I digested normal, sickle cell and sickle cell trait DNAs are consistent with our expected results. The DNA base sequence found in codons 5, 6, and 7 for the normal human β -globin gene are CCT, GAG, and GAG, with codon 6 being GTG in the β^S -allele. This single point mutation eliminates a *Dde* I recognition sequence (C-T-N-A-G, in which N is any base) in the β^S -allele, resulting in a new fragment of 376-bp as compared with 201-bp and 175-bp fragments found in normal individuals. In this study we describes the use of restriction endonuclease *Dde* I for the molecular analysis of the β^S -allele which was in accordance with the report given by Geever *et al.*^[9] Wilson *et al.*^[12] has been also used same enzyme for prenatal diagnosis of sickle cell anemia. But in their report an additional step of blot-hybridization was used which was very time-consuming.

In our report of PCR-RFLP analysis-is additional step of blot hybridization is not necessary and the analysis can be done directly with restriction digestion of the specific amplified DNA sequence. So it is a rapid and simple technique, one can use a number of restriction enzymes to find polymorphism of a restriction site adjacent to this gene.

In above result shows that majority of the population with positive PCR-RFLP were belonging to Kurmi (30%), Teli (39%) Rawat (9%), Gada (3%), Agharia (4%), Satnami (3%), Lohar (2%) and Bengali (3%) castes (Table1). Samples were selected randomly. The prevalence of the sickle cell disorder was found to be higher in female than male. It was significantly difference observed in sex wise prevalence of sickle cell disorder in chhattisgarh population. Ankushe^[11] reported prevalence to be 5.6 % from few villages of Wardha district. As regards to sex distribution of the disorder, Wintrobe^[12] stated that sickle cell trait is more common in female. But Samal *et al* did not find any such correlation. Deshmukh *et al.*^[20] in his cooperative study also had patients of sickle cell disorder which distributed more in higher

age groups. But in the present study we have found sickle cell disorder and sickle cell trait (Table 2) is more common in female and it increased with increasing age. Prevalence of sickle cell disorder is higher in Teli (39%), Kurmi (30%) and Rawat (9%) caste of Chhattisgarh population.

On the basis of above finding we can conclude that, RFLP analysis of the β -globin sequences in sickle cell patients from Chhattisgarh state shows a pattern similar to those reported in earlier studies from various parts of India.

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