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Microsatellite Diversity in HbS Carrier and Normal Individuals of Tribal Populations of Malaria Infested Regions

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KEY WORDS Sickle cell anemia; microsatellite markers; heterozygosity; Hardy-Weinberg equilibrium; genetic diversity.

ABSTRACT Sickle cell gene is a variant form of beta globin developed in human genome as a protective mechanism to malaria. The frequency of HbS gene varies considerably with ethnic group and geographical location coinciding with distribution of malaria. Occurrence of sickle cell mutation is very high among different endogamous groups of Chattisgarh, Maharastra, and Jharkhand state with equally high incidence of malaria infection. Hardy-Weinberg test was performed to assess genetic equilibrium among HbS carriers and normal individual of Agharia, Pawra and Oraon communities of the three states. Genetic diversity in two groups across fifteen microsatellite markers was studied using three important parameters viz., number of alleles, allele size variance and heterozygosity. Significant departure from Hardy Weinberg equilibrium was observed only at TH01 (11p15.5) among the carriers, which could be due to association of the locus with beta globin gene (11p15.5). Allele diversity is observed to be very high in normal individuals at each locus (with additional rare alleles at FGA, Penta D and D21S11 loci) in comparison to sickle cell carriers. The difference in the heterozygosity level (~5%) between two groups was observed to be highest at two significant loci THOI and CSF1PO (5q33.3-34), thereby indicating maximum pressure of the malarial parasite at these loci, leading to increase in the number of repeat units, among the normal individuals. Perhaps high genetic diversity in the normal group is to add resistant allele against malaria. Our study clearly demonstrates that microsatellites, besides being hot spots for recombination has another important role in enabling the individual to adapt to the stressful environment of parasite.

INTRODUCTION

The most common mutation in the beta globin gene that produces abnormal hemoglobin protein is a single base change in the DNA sequence. Sickle cell anemia is one of the most frequent of all abnormal hemoglobin variants developed in human genome as protective mechanism against dreadful malaria infection particularly in the equatorial belt. Hemoglobin-S gene is widespread among many endogamous tribal groups of India, like, Irular and Pallar (Tamilnadu-17.7% and 0.4%), Gond (Uttar Pradesh- 9.2%), Santhal (West Bengal-0.6%), Oraon, Ho, and Bhumij (Jharkhand-0.4%, 0.3%, 0.3%) and Mahadeo Koli (Maharastra-0.1%) (Undevia et al. 1981; Balgir 1996; Chaudhuri et al. 1967; Saha et al. 1998; Iswad and Naik 1984) inhabiting malaria infested regions.

Microsatellites are the most powerful marker for human diversity studies as their variation is thought to be selectively neutral with the exception of those STRs, which are tightly linked to or located inside a locus under selection (Slatkin 1995). Reports on their extensive use to identify people at risk for different disease (cancer, neurological disorders like, Schizophrenia, Huntington's disease, spinal bulbar muscular atrophy etc and malaria infection) suggest that they have a functional role also (Bailer et al. 2002; Rihet et al. 1998). The variation in microsatellite alleles is said to be adaptive in different environments (Maxon and Wills 1999), a short allele may be adaptive in one surrounding, and a long allele with many repeat units in a different milieu. Variation within the population would ensure the survival of the group in varying situation. Microsatellite markers selected for the present analysis demonstrate extensive polymorphism across global population hence validated for population genetics study (Budowle et al. 1999).

With these rationales a comparative account of allele distribution and genetic diversity across 15 STR markers among the sickle cell carriers and normal individuals of Agharia, Pawra and Oraon communities of Chattisgarh, Maharastra and Jharkhand state of India, who are under similar threat of malarial infection was carried out. The objectives are to: 1) to analyse the extent of genetic diversity across 15 STR loci among the foresaid groups, 2) to understand the difference in the genetic make up of sickle cell carriers and

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normal individuals, 3) since the selected communities are highly endogamous (reproductively isolated), therefore genetic equilibrium (Hardy-Weinberg) tests were performed to verify significant departure (if any) in the group, at any of the microsatellite loci. The levels of significance for each test statistic were evaluated through 1000 replicates of permutations of the observed alleles within each group.

Genetic diversity in terms of three important parameters - number of alleles, allele size variance and heterozygosity (if found) in the normal groups (both groups under same environmental stress of malaria parasite) will further substantiate the role of microsatellite in evolutionary adaptations towards combating the severe malarial infection. This is perhaps the first genetic study to understand the adaptive role of microsatellite markers against malaria infection in higher organisms, based upon fifteen STR markers, through comparative study of allele distribution pattern and genetic diversity among sickle cell carriers and normal individuals of the same communities facing equal threat of parasite.

MATERIALS AND METHODS

Clinical Samples: Total of 200 samples were collected from different communities of various states viz. Oraon (N=35) from Jharkhand, Pawara (N=100) of Maharastra, Agharia (N=65) of Chattisgarh state and were screened for sickle cell anemia (Table 1). The sociocultural and ethnic backgrounds of the populations are given in table 1.

Extraction and Polymerase Chain Reaction of Genomic DNA: The standard protocol of organic extraction (Sambrook et al. 1998) was used for the isolation of DNA. Sickle Cell Anemia was detected using PCR-RFLP method. Sickle cell mutation abolishes a restriction endonuclease site (Dde-I), and electrophoretic resolution of the fragment pattern reveals the presence or absence of the mutation. The following primers were utilized for amplification of the exon-1 of the beta globin gene (where the point mutation of sickle cell anemia is found).

1. T1946/F (5′ ACCTCACCCTGTGGAGCCAC-3′)
2. T1947/R (5′ GAGTGGACAGATCCCCAAAGGACTCAAAGA-3′)

PCR cocktail of 12.5 µl comprised of 0.4 pmol primers, 2 µM Mg Cl_2; 2.0 µl (10x buffer); 200 µM dNTP; 2.0 units of Taq polymerase enzyme; and 10-50ng of purified human genomic DNA was set for amplification in thermalcycler program: Denatured for 5min at 94°C, followed by 30 cycles of 94°C for 0.30 min, 65°C for 1.00 min, and 70°C for 1.30 min, and an extension of 5.00 min at 70°C. The amplified products is of 442 bp size. PCR products were digested for 3 hours with Dde-I, with appropriate buffers and incubation temperature. The digested products were 375- and 67-bp fragments in sickle cell anemia (S/S), 220-, 155- and 67-bp in normal individuals. Carriers have fragment sizes of 375-, 220-, 155- and 67-bp.

Microsatellite Markers and Genotyping: Markers selected for the present study are highly polymorphic short tandem repeat (STR) sequences of tetra- and pentanucleotides, with large number of co-dominant alleles, due to high mutation and recombination rates (~1/1000) (Weber and Wong 1993). Details of the chosen microsatellites (chromosomal location, core repeat sequence 5′→3′ and repeat numbers of allelic ladder components) are presented in table 2. The co-amplification of the 15 highly polymorphic microsatellite markers was performed using 12.5ul final volume containing 1-5 ng of template and reagents supplied with the kit (Promega Corporation, Madison, U.S.A). Genotyping was done in ABI Prism™ 377 DNA Sequencer. The fragment data analysis and allele designation were carried out using the Genescan ™ and Genotyper ™ softwares.

Statistical Analysis: As the studied loci are autosomal co-dominant, frequency of each allele for individual STRs were calculated from the numbers of each genotype in the sample set by method of gene count. The unbiased estimates of the expected heterozygosity were computed

Table1: Population groups, location and size of samples, ethnicity, sociocultural and occupational backgrounds of communities harboring HbS gene in India

Populations	*Sample size*	*Sampling location*	*Sociocultural affiliation*	*Ethnicity*	*Linguistic affiliation*
Agharia	65	Chattisgarh	Agriculture, Backward caste	Indo-Caucasoid	Indo-European
Pawra	100	Maharastra	Agriculture, Tribe	Indo-Austroloid	Indo-European
Oraon J. Traibes	35	Jharkhand	Agriculture, Tribe	Indo-Austroloid	Austro-Asiatic

(Edward et al. 1992). Possible divergence from the Hardy-Weinberg expectations was determined by calculating the unbiased estimates of the expected homozygote, heterozygote frequencies (Chakraborty et al. 1998; Nei and Roychaoudhary 1974; Nei 1978) likelihood ratio tests (Chakraborty et al. 1991; and Weir 1992) and the exact test (Guo and Thompson 1992). Lastly the allele size variance was calculated through computer simulating programmer MICROSAT (http://lotka.stanford.edu/microsat.html)

RESULTS AND DISCUSSION

Hemoglobinopathy like sickle cell anemia occurs both in heterozygous (called as sickle cell trait) and homozygous forms. Prevalence of HbS trait in areas hyperendemic for malaria, suggests that HbS gene in heterozygous condition confers selective protection against lethal forms of malaria (Haldane 1949). Sickle cell trait is observed to be 28%, 16% and 22% among Agharia, Pawara, and Oraon communities of Chattisgarh, Maharastra and Jharkhand where incidences of *P. falciparum* and *P. vivax* malaria is also very high. Further study on sickle cell trait and normal individuals of these communities was carried out with the objective to see the differences in the genome caused by the deleterious effect of the malarial parasite. The differences here refer to the number of alleles, allele size variance and the level of heterozygosity across 15 microsatellite markers, which are spread over 13 different chromosomes, so as to scan maximum part of the genome.

Three tests viz., exact test for multiallelic loci, log likelihood method and the homozygosity test along with allele frequencies by gene counting method were performed for testing concord with the Hardy-Weinberg proportion of genotype frequencies among sickle cell carriers and the normal group. No detectable deviation from genetic equilibrium across any of the 15 loci in the two groups were observed except at TH01 among the carriers, where the exact test value is below 5% level of significance (0.038). Exact test is the most powerful of the three foresaid analysis procedure (Chakraborty, 1994) and therefore disequilibrium / deviation in the population at TH01 locus assumes significance. Microsatellite marker TH01 (intron-1 of human tyrosine hydroxylase gene) is located very close (11p15.5) to the beta globin gene (11p15.5), which might be the cause of departure from equilibrium among the disease group. The locus wise values of allele frequencies, exact test, likelihood estimate and homozygosity test are shown in table 3 and table 4 of the sickle cell heterozygotes and normal groups respectively.

The allele frequencies observed in the HbS group (Table 3) indicate moderately large number

Table 2: Locus specific information of 15 microsatellite markers analysed in HbS carriers and normal individuals of malaria infested regions

STR Locus	*Chromosomal location*	*Repeat sequence*	*Total number of alleles*	*Reported alleles*
vWA	12p12-pter	TCTA Complex (19)	13	10-22
D8S1179	8q	TCTA Complex (19)	12	7-18
TPOX	2p23-2pter	AATG	8	6-13
FGA	4q28	TTTC Complex (19)	28	16-18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26-30, 31.2, 43.2, 44.2, 45.2, 46.2
D5S818	5q23.3-32	AGAT	10	7-16
D13S317	13q22-q31	TATC	9	7-15
D7S820	7q11.21-22	GATA	9	6-14
D16S539	16q24-qter	GATA	9	5, 8-15
CSF1PO	5q33.3-34	AGAT	10	6-15
Penta D	21q	AAAGA	14	2.2, 3.2, 5, 7-17
D3S1358	3p	TCTA Complex	9	12-20
TH01	11p15.5	AATG (19)	10	4 - 9, 9.3, 10-11, 13.3
D21S11	21q11-21q21	TCTA Complex (19)	25	24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32,32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38
D18S51	18q21.3	AGAA (19)	22	8-10, 10.2, 11-13, 13.2, 14-27
Penta E	15q	AAAGA	20	5-24

Table 3: Allele frequencies and HW statistical parameters of 15 STR loci of Sickle cell populations* (N=72) inhabiting malaria endemic states of India

Allele	*vWA*	*D8S1179*	*TPOX*	*FGA*	*D5S818*	*D13S317*	*D7S820*	*D16S539*	*CSF1PO*	*Penta-D*	*D3S1358*	*THO1*	*D21S11*	*D18S51*	*Penta-E*
3.2	-	-	-	-	-	-	-	-	-	0.007	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	0.007	-	-	-
5	-	-	-	-	-	-	-	-	-	0.007	-	0.01	-	-	0.042
6	-	-	-	-	-	-	-	-	-	-	-	0.214	-	-	-
7	-	0.007	-	-	0.007	0.014	0.007	-	-	0.007	-	0.107	-	-	0.028
8	-	0.007	0.271	-	-	0.243	0.278	0.035	-	0.007	-	0.2	-	-	-
9	-	0.007	0.25	-	0.076	0.063	0.132	0.174	0.014	0.229	-	0.336	-	-	0.021
9.3	-	-	-	-	-	-	-	-	-	-	-	0.121	-	-	-
10	-	0.16	0.057	-	0.146	0.09	0.215	0.104	0.127	0.166	-	0.007	-	0.014	0.028
11	-	0.09	0.407	-	0.188	0.215	0.208	0.298	0.268	0.236	-	-	-	0.021	0.234
12	-	0.063	0.014	-	0.34	0.278	0.125	0.222	0.478	0.125	0.007	-	-	0.139	0.141
13	0.01	0.16	-	-	0.215	0.076	0.021	0.153	0.099	0.153	0.14	-	-	0.146	0.077
14	0.289	0.236	-	-	0.021	0.021	0.014	0.014	0.014	0.021	0.34	-	-	0.229	0.056
15	0.099	0.181	-	-	0.007	-	-	-	-	0.042	0.208	-	-	0.194	0.049
16	0.141	0.069	-	-	-	-	-	-	-	-	0.222	-	-	0.111	0.092
17	0.239	0.021	-	-	-	-	-	-	-	-	0.076	-	-	0.069	0.106
18	0.155	-	-	-	-	-	-	-	-	-	0.007	-	-	0.014	0.077
19	0.049	-	-	-	-	-	-	-	-	-	-	-	-	0.021	0.028
19.2	-	-	-	0.023	-	-	-	-	-	-	-	-	-	-	-
20	0.014	-	-	0.099	-	-	-	-	-	-	-	-	-	0.028	0.021
20.2	-	-	-	0.023	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	0.054	-	-	-	-	-	-	-	-	-	0.014	-
21.2	-	-	-	0.058	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	0.117	-	-	-	-	-	-	-	-	-	-	-
22.2	-	-	-	0.023	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	0.149	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	0.122	-	-	-	-	-	-	-	-	-	-	-
24.2	-	-	-	0.054	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	0.14	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	0.023	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	0.081	-	-	-	-	-	-	-	-	0.014	-	-
28	-	-	-	0.018	-	-	-	-	-	-	-	-	0.085	-	-
28.2	-	-	-	-	-	-	-	-	-	-	-	-	0.007	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	0.302	-	-
29.2	-	-	-	-	-	-	-	-	-	-	-	-	0.007	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	0.176	-	-
30.2	-	-	-	-	-	-	-	-	-	-	-	-	0.028	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-	0.035	-	-
31.2	-	-	-	0.018	-	-	-	-	-	-	-	-	0.141	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	0.007	-	-
32.2	-	-	-	-	-	-	-	-	-	-	-	-	0.113	-	-
33.2	-	-	-	-	-	-	-	-	-	-	-	-	0.085	-	-
HP	0.279	0.091	0.195	0.66	0.279	0.244	0.99	0.928	0.632	0.147	0.318	0.446	0.227	0.838	0.006
ET	0.96	0.099	0.793	0.477	0.063	0.103	0.695	0.822	0.694	0.053	0.831	0.038	0.776	0.841	0.385
LR	0.869	0.157	0.227	0.347	0.05	0.092	0.396	0.82	0.56	0.068	0.88	0.056	0.693	0.885	0.428

HP: Probability of homozygosity; ET: Exact test; LR: Likelihood ratio
*populations: Agharia (N=65), Pwara (N=100) and Oraon (N=35)

of alleles at six loci namely Penta E, FGA, D18S51, D21S11, Penta D and D8S1179. The expressed number of alleles in the carrier group ranges from 5, at the locus TPOX (least polymorphic) to 15 and 14, at FGA and Penta E loci (most polymorphic). However, normal individuals of the selected three communities (Agharia, Pawra and Oraon) exhibit a different pattern of allele distribution (Table 4) than sickle cell carriers. Number of alleles range from 7, at TPOX and

Table 4: Allele frequencies and HW statistical parameters of 15 STR loci of normal individuals (N=128) of population* inhabiting malaria endemic states of India

Allele	*vWA*	*D8S1179*	*TPOX*	*FGA*	*D5S818*	*D13S317*	*D7S820*	*D16S539*	*CSF1PO*	*Penta-D*	*D3S1358*	*THO1*	*D21S11*	*D18S51*	*Penta-E*
4	-	-	-	-	-	-	-	-	-	-	-	0.004	-	-	-
5	-	-	0.002	-	-	-	-	-	-	0.004	-	0.004	-	-	0.056
6	-	-	-	-	-	-	-	-	-	0.004	-	0.305	-	-	-
7	-	-	0.011	-	0.002	0.017	0.023	-	0.002	0.01	-	0.152	-	-	0.062
8	-	0.009	0.347	-	0.004	0.184	0.21	0.07	0.002	———	-	0.155	-	0.002	0.004
9	-	0.005	0.14	-	0.053	0.088	0.137	0.134	0.02	0.226	-	0.283	-	-	0.01
9.3	-	-	-	-	-	-	-	-	-	-	-	0.068	-	-	-
10	-	0.154	0.101	-	0.121	0.105	0.203	0.115	0.219	0.22	-	0.027	-	0.014	0.033
10.2	-	-	-	-	———	———	———	———	———	———	-	-	-	0.002	-
11	0.004	0.092	0.35	-	0.31	0.29	0.237	0.351	0.269	0.239	-	0.002	-	0.026	0.071
12	0.006	0.1	0.05	-	0.361	0.275	0.149	0.213	0.384	0.107	0.009	-	-	0.118	0.071
13	0.002	0.197	-	-	0.149	0.058	0.037	0.117	0.095	0.088	0.009	-	-	0.107	0.106
13.2	-	-	-	-	-	-	-	-	-	-	-	-	-	0.007	-
14	0.171	0.208	-	-	0.009	0.014	0.004	0.011	0.007	0.064	0.041	-	-	0.235	0.056
15	0.117	0.146	-	-	0.006	0.002	———	———	0.002	0.027	0.318	-	-	0.181	0.144
16	0.187	0.065	-	-	-	-	-	-	-	0.007	0.294	-	-	0.094	0.1
17	0.284	0.024	-	-	-	-	-	-	-	0.004	0.249	-	-	0.098	0.101
17.2	-	-	-	0.003	-	-	-	-	-	-	-	-	-	-	-
18	0.137	0.006	-	0.009	-	-	-	-	-	-	0.057	-	-	0.044	0.048
18.2	-	-	-	0.003	-	-	-	-	-	-	-	-	-	-	-
19	0.072	-	-	0.055	-	-	-	-	-	-	0.016	-	-	0.04	0.098
19.2	-	-	-	0.003	-	-	-	-	-	-	-	-	-	-	-
20	0.014	-	-	0.069	-	-	-	-	-	-	0.007	-	-	0.004	0.018
20.2	-	-	-	0.028	-	-	-	-	-	-	-	-	-	-	-
21	0.004	-	-	0.126	-	-	-	-	-	-	-	-	-	0.002	0.018
21.2	-	-	-	0.016	-	-	-	-	-	-	-	-	-	-	-
22	0.002	-	-	0.118	-	-	-	-	-	-	-	-	-	0.009	-
22.2	-	-	-	0.012	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	0.153	-	-	-	-	-	-	-	-	-	-	-
23.2	-	-	-	0.024	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	0.157	-	-	-	-	-	-	-	-	-	0.011	0.004
24.2	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	0.118	-	-	-	-	-	-	-	-	-	-	-
25.2	-	-	-	0.009	-	-	-	-	-	-	-	-	0.002	-	-
26	-	-	-	0.043	-	-	-	-	-	-	-	-	-	0.002	-
27	-	-	-	0.025	-	-	-	-	-	-	-	-	0.016	0.004	-
27.2	-	-	-	-	-	-	-	-	-	-	-	-	0.004	-	-
28	-	-	-	0.003	-	-	-	-	-	-	-	-	0.14	-	-
28.2	-	-	-	-	-	-	-	-	-	-	-	-	0.004	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	0.238	-	-
29.2	-	-	-	-	-	-	-	-	-	-	-	-	0.012	-	-
30	-	-	-	0.003	-	-	-	-	-	-	-	-	0.155	-	-
30.2	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-	0.036	-	-
31.2	-	-	-	0.003	-	-	-	-	-	-	-	-	0.095	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	0.004	-	-
32.2	-	-	-	-	-	-	-	-	-	-	-	-	0.182	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	0.002	-	-
33.2	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-
34.2	-	-	-	-	-	-	-	-	-	-	-	-	0.004	-	-
36	-	-	-	-	-	-	-	-	-	-	-	-	0.002	-	-
38	-	-	-	-	-	-	-	-	-	-	-	-	0.004	-	-
HP	0.455	0.502	0.335	0.829	0.594	0.084	0.081	0.375	0.733	0.474	0.268	0.261	0.788	0.725	0.677
ET	0.136	0.215	0.387	0.277	0.446	0.472	0.444	0.523	0.478	0.528	0.06	0.49	0.401	0.383	0.455
LR	0.175	0.338	0.381	0.385	0.524	0.416	0.498	0.55	0.488	0.615	0.094	0.47	0.381	0.364	0.643

D16S539 to 22, at FGA which is much more higher than the carriers, indicating greater genetic diversity in the normal group. Since the number of segregating alleles is sensitive to sample size therefore other parameters, viz., allele size variance and heterozygosity, (Nei 1978; Kimmel et al. 1996) were calculated (Table 5) to substantiate the above observation. The values of allele size variance ranges from 0.87 (CSF1PO) to 12.14 (Penta E) among the carriers, and from 1.44 (D5S818) to 15.95 (Penta E) in the normal group. Maximum difference at Penta E locus (3.80) between two groups clearly depicts comparatively greater diversity in the normal group. Similar pattern had been observed when heterozygosity values were compared. The difference in the heterozygosity level between HbS gene carriers and normal group was found to be highest (~5%) at CSF1PO and TH01 marker. Locus CSF1PO (human c-fms proto-oncogene for CSF-1 receptor gene) is contiguous (5q33.3-34) to the locus 5q31-33 where genes implicated in the regulation of immune response to *Plasmodium falciparum* infection (Rihet 1998) are reported. Therefore high heterozygosity at these two loci is worth mentioning as it clearly indicates pressure of the malarial parasite is highest at TH01 and CSF1PO loci in normal host to add new allele for protection against deadly infection.

Table 5: Summary statistics of sickle cell and normal individuals at 15 STR loci

Loci	*Disease status*	*Allele number*	*Hetero-zygosity*	*Allele size variance*
vWA	Sickle cell carrier	8	0.8	2.93
	Normal	12	0.82	2.93
D8S1179	Sickle cell carrier	11	0.84	4.34
	Normal	11	0.85	4.16
TPOX	Sickle cell carrier	5	0.7	1.68
	Normal	7	0.74	2.11
FGA	Sickle cell carrier	15	0.87	6.9
	Normal	22	0.87	4.94
D5S818	Sickle cell carrier	8	0.78	1.79
	Normal	9	0.76	1.44
D13S317	Sickle cell carrier	8	0.8	3.29
	Normal	9	0.8	2.73
D7S820	Sickle cell carrier	8	0.8	2.42
	Normal	8	0.81	2.47
D16S539	Sickle cell carrier	7	0.8	2.08
	Normal	7	0.79	2.07
CSF1PO	Sickle cell carrier	6	0.67	0.87
	Normal	9	0.72	1.15
Penta D	Sickle cell carrier	11	0.82	3.55
	Normal	12	0.82	3.13
D3S1358	Sickle cell carrier	7	0.77	1.5
	Normal	9	0.75	1.55
TH01	Sickle cell carrier	8	0.69	1.63
	Normal	9	0.74	1.85
D21S11	Sickle cell carrier	12	0.8	2.22
	Normal	18	0.83	3.15
D18S51	Sickle cell carrier	12	0.85	4.57
	Normal	19	0.87	7.44
Penta E	Sickle cell carrier	14	0.88	12.14
	Normal	17	0.92	15.95

The present study thus, clearly demonstrates that although the two groups (sickle cell gene carriers and normal) comprise of same communities, the level of heterozygosity, observed range of alleles and the allele size variance which are three basic and vital measures of genetic variation is much more higher among the normal set than the HbS heterozygotes at all of the selected loci (except three namely D5S818, Penta D and D3S1358 where the heterozygosity value of the two groups is almost same or the difference is statistically insignificant). Since both groups are equally exposed to the stress of malaria parasite, the presence of large number of alleles including some additional rare alleles (allele-17.2 of FGA, allele-6 of Penta E and allele-27.2 of D21S11), clearly illustrates that exposure to the malarial parasite trauma has generated pressure on the host genome causing diversification of alleles (by generating new alleles) to add new and better resistant allele, in order to stay alive, as an alternative protective mechanism to sickle cell mutation against dreadful infection. Low allele diversity among the heterozygotes could be because of the HbS gene that restricts the proliferation of the parasite in the red blood cell, reducing the parasite load, thus less distress to host genome. Our study thus substantiates that microsatellites have a definite role in adaptation towards infectious disease like malaria, besides being hot spot for recombination. The genetic profile of sickle cell trait and normal individuals based upon 15 microsatellite markers can also be used to understand the history and demography of the disorder.

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