J Community Genet (2011) 2:147–151
DOI 10.1007/s12687-011-0050-4

ORIGINAL ARTICLE

Screening for the sickle cell gene in Chhattisgarh state, India: an approach to a major public health problem

Pradeep K. Patra · Virander S. Chauhan · Prafulla K. Khodiar · Abdul R. Dalla · Graham R. Serjeant

Received: 8 February 2011 / Accepted: 13 May 2011 / Published online: 15 June 2011

Abstract The aim of this study is to determine the feasibility of large-scale population screening for the sickle cell gene in high risk areas with limited resources. A programme designed to detect the sickle cell trait and sickle cell disease has screened 359,823 subjects among 2,087 (99.7%) of the villages in Raipur District, Chhattisgarh State, India between October 2007 and June 2010. Children aged 3–15 years were initially screened in the villages by solubility tests on fingerprick samples. Venipuncture was performed on subjects with positive solubility tests, and the samples were transferred to Raipur Medical College for alkaline haemoglobin electrophoresis. The sickle cell trait occurred in 33,467 (9.30%) and an SS phenotype in 747 (0.21%). The gene frequencies were not in Hardy–Weinberg equilibrium most likely due to a deficiency of the SS phenotype failing to enter the sampled population from either sickness or early death. Subjects with abnormal haemoglobin genotypes may factor this information into decisions regarding marriage and avoid the risks of having children with sickle cell disease. The techniques described may be a model for other developing societies with limited resources.

Keywords Population screening · Sickle cell trait · Sickle cell disease · India

P. K. Patra · P. K. Khodiar
Department of Biochemistry, Pt. J.N.M. Medical College, Raipur, Chhattisgarh, India

V. S. Chauhan
ICGEB,
New Delhi, India

A. R. Dalla
India Red Cross Society,
Raipur, Chhattisgarh, India

G. R. Serjeant (✉)
Sickle Cell Trust (Jamaica),
14 Milverton Crescent,
Kingston, Jamaica
e-mail: grserjeant@cwjamaica.com

Introduction

For many years, the sickle cell gene has been considered confined to people of African ancestry although the gene was described in southern India, in persons without African origin, as early as 1952 (Lehmann and Cutbush 1952). This paper described HbS among the Paniyan, Kurumba, Mullakurumba and Irula tribes in mountainous regions in the north of Tamil Nadu, an observation initially considered to be only of anthropological interest. However, over the next 30 years, population screening and especially the Anthropological Survey of India (Negi 1972) established sickle cell trait frequencies up to 35% throughout much of central India, the highest frequencies occurring in Orissa, Madhya Pradesh, Maharastra and Gujarat (Balgir 1996; Ambedkar et al. 2001). A smaller focus of the gene occurred in southern India in the north of Kerala and Tamil Nadu. The sickle cell gene occurs throughout Indian society although it is most common among the tribal people, who are predominantly agricultural and often live in remote areas, presenting particular problems in provision of health care and education. Chhattisgarh is a new state, formed in November 2000 from the south-eastern part of Madhya Pradesh, with a population of approximately 21 million of whom 32% are tribal in origin. The capital is Raipur and the medical college at Raipur has been conducting surveys for the sickle cell gene in the surrounding areas since 2007. The procedures used and results obtained are presented

since these may be of interest to other groups working in areas with high prevalence of the sickle cell gene.

Material and methods

Subjects Of the estimated 2,094 villages within Raipur District, 2,087 (99.7%) were screened between October 2007 and June 2010. The focus group were children aged 3–15 years, and after discussion with the village heads and the school headmaster, written consent was obtained from the parents or village heads on behalf of the community. The screening teams, comprising a doctor, supervisor, counsellor and two laboratory technologists, worked in schools or village community halls and returned each evening to the base (Biochemistry Laboratories of the Pandit Jawaharlal Nehru Medical College, Raipur). The villages covered were within 80–100 km from Raipur, and depending on the location of the villages, the teams screened an average of 500–600 subjects daily.

Technical procedures Fingerprick samples were taken by the technologists into glass tubes containing reagents (hyperosmolar phosphate buffer, saponin, reducing agent) for the solubility test, and all positive subjects had 5 ml of venous blood taken into tubes containing EDTA, stored in the cool until return to the base laboratory. Alkaline haemoglobin electrophoresis on cellulose acetate in Tris–EDTA–borate buffer at pH 8.6 was conducted on all solubility positive samples along with known controls.

Genotype results and counselling

Subjects with abnormal genotypes were given cards displaying name, age/date of birth, village/school and genotype AS/SS and a single page leaflet with simple information on the importance of premarital testing of prospective partners. The abnormal genotype cards were distributed by the counsellor who gave additional information and answered questions. Subjects with SS disease received an eight-page illustrated booklet containing information on disease-related problems, inheritance patterns, and were encouraged to visit the local primary health centre or community health centre. No cards were given to subjects with a presumed normal AA haemoglobin phenotype.

Social classification The nomenclature was derived from Articles 340(1) and 340(2) of the Constitution of India. General castes include the four categories of the Indian caste system (Brahmin, Kshatriyas, Vaishas and Shudras). Scheduled castes (SC) traditionally occupy the lowest status in Indian society, were previously known as the 'untouchables' and now officially referred to as 'Harijans' or 'Dalits'. Scheduled tribes (ST) are tribal communities which have been declared as such by the President through public notification. They are widespread but occur mainly in forest and hilly regions, are often geographically isolated, shy of other human contact, economically backward and outside the Indian caste system. Other backward classes (OBC) are defined in the Constitution of India as 'socially and educationally backward classes' and falling outside the definitions of scheduled castes and tribes, these are also targeted for special programmes of social and educational advancement. The relative proportions of these social groups in the State of Chhattisgarh are general castes (17%), SC (25%), ST (8%) and OBC (50%).

Results

A total of 359,823 subjects were screened detecting 33,467 (9.30%) with the sickle cell trait and 747 (0.21%) with a phenotype consistent with homozygous sickle cell disease (Table 1). These frequencies deviate significantly ($p=0.0002$) from that expected from the Hardy–Weinberg equilibrium, there being a projected deficiency of SS subjects (observed 747, expected 849). The highest frequency of the sickle cell gene occurred among the scheduled tribes (Halba 16%, Gond 13% and Binjhwar 11%), scheduled castes (Ghasia 24%, Ganda 22% and Mahar 12%) and the other backward classes (Agharia 19%, Kolta 17%, Kurmi 16%, Teli 15% and Kumhar 10%). Analysis of age and gender distribution (Table 2) showed that age group 3–6 years had smaller representation (1.0–6.5% for each year age band) compared with age groups 7–15 years (7.4–12.0% for each year age band). The sickle cell trait prevalence increased significantly with age (incidence rate ratio=1.012, $p<0.001$) but no age-related trend occurred with SS disease.

Discussion

The frequency of the sickle cell trait among the scheduled tribes and other backward classes in Raipur District of Chhattisgarh State exceeded 10% similar to the 11.1% reported from the adjacent western Orissa (Kar 1991), but less than the 16.6% reported among children aged up to 15 years in south Orissa (Sahu et al. 2003). An analysis of Orissan subjects admitted to the Burla Medical College in western Orissa revealed an average frequency of the sickle cell trait of 12.2% but also drew attention to the wide variation in frequency between different social groups varying from 1.3% among Brahmins to 20% among the scheduled castes (Kar et al. 1987). The frequency of 29% among the Agharias of the warrior caste is an anomaly

Table 1 Distribution of genotypes among 359,823 subjects in Raipur district

	Total	AA		AS		SS	
		n	(%)	*n*	(%)	*n*	(%)
Number of subjects							
Males	177,378	160,664	(90.58)	16,343	(9.21)	371	(0.21)
Females	182,445	164,945	(90.41)	17,124	(9.39)	376	(0.21)
Total	359,823	325,609	(90.49)	33,467	(9.30)	747	(0.21)
Social category							
General caste	29,152	27,464	(94.21)	1,648	(5.65)	40	(0.14)
Scheduled caste	65,177	60,043	(92.12)	4,996	(7.67)	138	(0.21)
Scheduled tribe	45,453	41,131	(90.49)	4,228	(9.30)	94	(0.21)
OBC	220,041	196,971	(89.52)	22,595	(10.27)	475	(0.22)
Total	359,823	325,609	(90.49)	33,467	(9.30)	747	(0.21)

which has been previously reported (Samal et al. 1978; Samal and Naik 1983). The effect of social grouping on the frequency of the sickle cell trait was also noted in the present study with the highest frequency occurring among the scheduled tribes and OBCs of Raipur District. The relevance of these observations is that sickle cell disease is a major contributor to the health problems of these populations and that the high frequency of the sickle cell trait raises the possibility of prevention of the disease through genotype detection and counselling.

The current study illustrates that genotype detection is feasible on a substantial scale with limited resources. Screening by the solubility test with confirmation of genotype by alkali haemoglobin electrophoresis targets only the HbS gene, and although this is clearly the dominant abnormal haemoglobin in central India, it will not detect the less frequent abnormal haemoglobins such as HbD Punjab or HbE. A more serious defect is the inability to detect the beta thalassaemia trait which is also widespread in India, the prevalence among tribal groups averaging 6–18% depending on sample selection and geographic area (Balgir 2005a, 2005b; Choubisa 2009). Two tribes, the Kharia and Bhuyan in Sundergarh District of Orissa adjacent to Chhattisgarh, were found to have beta thalassaemia trait frequencies of 6–7% (Balgir 2005b) which may also be representative of the tribal people in Chhattisgarh State. Detection of the beta thalassaemia gene requires haematological indices and confirmation by HbA_2 levels in subjects with low MCV and MCH, and would greatly increase the complexity of the screening programme but with a relatively high frequency of beta thalassaemia trait among tribal people; this must be accepted as a

Table 2 Age and gender distribution of whole group, sickle cell trait (AS) and homozygous sickle cell (SS) disease

Age group (years)	Whole group			AS			SS		
	Male	Female	Total	Male	Female	Total (%)	Male	Female	Total (%)
3	1,751	1,762	3,513	165	144	309 (8.80)	10	4	14 (0.40)
4	3,110	2,949	6,059	294	257	551 (9.09)	9	15	24 (0.40)
5	4,104	3,861	7,965	368	324	692 (8.69)	8	14	22 (0.28)
6	11,860	11,449	23,309	996	993	1,989 (8.53)	17	24	41 (0.18)
7	14,267	14,097	28,364	1,261	1,255	2,516 (8.87)	29	34	63 (0.22)
8	17,033	16,362	33,395	1,454	1,456	2,910 (8.71)	32	31	63 (0.19)
9	18,435	18,288	36,723	1,679	1,689	3,368 (9.17)	38	41	79 (0.22)
10	21,614	21,655	43,269	2,092	2,083	4,175 (9.65)	34	26	60 (0.14)
11	19,400	19,986	39,386	1,818	1,948	3,766 (9.56)	32	40	72 (0.18)
12	20,763	21,187	41,950	1,973	2,046	4,019 (9.58)	56	42	98 (0.22)
13	16,735	18,711	35,446	1,548	1,746	3,294 (9.29)	46	39	85 (0.24)
14	15,939	17,718	33,657	1,524	1,751	3,275 (9.73)	40	36	76 (0.23)
15	12,367	14,420	26,787	1,171	1,432	2,603 (9.72)	20	30	50 (0.19)
Total	177,378	182,445	359,823	16,343	17,124	33,467 (9.30)	371	376	747 (0.21)

The age-related trend in AS and SS births was tested using Poisson regression, adjusting for sex and including age as a continuous term

shortcoming of the present population screening programme. Two proposals which could contribute useful knowledge would be a detailed study of a sample of the population to learn the frequency of the beta thalassaemia trait in this population and also to offer these tests to the prospective partners of sickle cell trait carriers prior to marriage. This would then inform the couple of their reproductive options. The lack of further study also limits genotypic precision in subjects found to have the SS phenotype which, without further investigation and family studies, will fail to distinguish subjects with sickle cell-hereditary persistence of fetal haemoglobin, sickle cell-beta^{o} thalassaemia and the severe Indian sickle cell-beta^{+} thalassaemia due to the IVS1-5 G→C mutation. An analysis of 700 patients with sickle cell disease at Burla Medical College in western Orissa estimated that 57 (8.1%) had sickle cell-beta thalassaemia (Kar 1991), implying that this genotype may have accounted for 60 subjects with an SS phenotype in the present study. However, the majority of these cases are likely to be homozygous sickle cell (SS) disease and this lack of precision is a compromise necessary in programmes capable of screening large populations.

The relative deficiency of the SS phenotype compared with the frequency of the sickle cell trait indicates that some cases fail to enter the screened population either as a result of early death, failure to register at school or illness on the day of screening. In most SS populations of African origin, there is a high early mortality and continued attrition with age but the Asian haplotype which characterises SS disease in the Indian subcontinent has mild features and is also linked to high levels of fetal haemoglobin and frequent alpha thalassaemia, both likely to ameliorate the disease. An increasing frequency of the sickle cell trait with age also remains unexplained, since any protective effect of the trait against malaria is unlikely to operate after the age of 3 years. Possible interpretations are the preferential selection of subjects in families known to have sickle cell disease among the older subjects or a real decrease of the sickle cell trait with secular year in the sampled population because of social changes such as greater admixture of high and low trait frequency populations.

Crucial to the success of this programme is the education and counselling delivered to carriers of abnormal genotypes. Even in established relationships where both parents have the sickle cell trait, the chances of a child with SS disease are only 1 in 4 for each pregnancy, implying that there is a 75% chance of a healthy child. Another option becoming more widely available and acceptable in India is the role of prenatal diagnosis in which fetal material, either from the chorion or amniotic fluid, can be used to ascertain the genotype of the fetus at 12–14 weeks of pregnancy, allowing the option of termination if desired. Influencing the decision on termination will be knowledge of the natural history of SS disease in India which is currently undocumented. There is increasing evidence that the disease in India differs from that in Africa, a milder clinical course resulting from the high fetal haemoglobin levels and frequent alpha thalassaemia characteristic of Indian disease. Documentation of Indian sickle cell disease should be based on newborn screening which has the added advantage of identifying the population at risk of early complications, many of which can be prevented by proper prospective care. Newborn screening for sickle cell disease in India is currently at the stage of pilot projects but the extensive experience available from elsewhere indicates that it is readily achieved and must be accompanied by close follow-up of the detected babies. The establishment of extensive newborn screening should be an urgent priority, not only for management of the disease but for documenting its clinical course in India.

Within the limitations discussed above, the present study has screened over one third of a million subjects detecting those heterozygous and probably homozygous for the sickle cell gene. It is clear that this is a major haemoglobinopathy among the tribal people of Chhattisgarh, and the education and counselling given to carriers of the HbS gene may influence arranged marriages and consequently reproductive decisions. Those detected to have probable SS disease require clinical documentation, further study and management protocols which must be disseminated through the local health centres. This group provides major challenges for management but the State of Chhattisgarh is well placed to develop protocols which could be implemented throughout the affected populations of India.

Acknowledgments We thank the Government of Chhattisgarh for the financial, infrastructural support and cooperation and the Department of Biotechnology (DBT), Government of India for the financial support. We also thank Prof. Ian Hambleton, Chronic Disease Research Unit, Tropical Medicine Research Institute, University of the West Indies, Bridgetown, Barbados for some of the statistical analyses.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Ambedkar SS, Phadke MA, Mokashi GD, Bankar MP, Khedkar VA, Venkat V, Basutkar DG (2001) Pattern of haemoglobinopathies in western Maharastra. Indian Pediatr 38:530–534

Balgir RS (1996) Genetic epidemiology of the three predominant abnormal haemoglobins in India. J Assoc Physicians India 44:25–28

Balgir RS (2005a) Spectrum of hemoglobinopathies in the state of Orissa, India: a ten years cohort study. J Assoc Physicians India 53:1021–1026

Balgir RS (2005b) The spectrum of haemoglobin variants in two scheduled tribes of Sundargarh district in north-western Orissa, India. Ann Hum Biol 32:560–573

Choubisa SL (2009) Sickle cell haemoglobin, thalassaemia and G-6-PD enzyme deficiency genes in Garasiya tribe inhabited malaria endemic areas of Sirohi District, Rajasthan (India). J Commun Dis 41:13–18

Kar BC (1991) Sickle cell disease in India. J Assoc Physicians India 39:954–960

Kar BC, Devi S, Dash KG, Das M (1987) The sickle cell gene is widespread in India. Trans R Soc Trop Med Hyg 81:273–275

Lehmann H, Cutbush M (1952) Sickle-cell trait in southern India. Br Med J 1:404

Negi RS (1972) Sickle cell trait in India. A review of known distribution. Bull Anthropol Surv India 17:439–449

Sahu T, Sahani NC, Das S (2003) Sahu SK (2003) Sickle cell anaemia in tribal children of Gajapati district in south Orissa. Indian J Community Med 28:11–14

Samal GC, Naik KN (1983) Incidence of sickle-cell disease in school children of western Orissa. Ind J Haematol 1:51–54

Samal GC, Ahmed B, Behera SK (1978) Incidence of sickle-cell disease. Pediatr Clin India 13:33–36