

Blood Groups, Hemoglobinopathy and G-6-PD Deficiency Investigations Among Fifteen Major Scheduled Tribes of Orissa, India

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INTRODUCTION

Tribal communities especially the primitive tribes in India share the largest tribal population in the world. As per 1991 Census, there were 461 tribal groups which constituted 8.08% (about 68 million) of the total population of India. Out of these, as many as 62 tribes live in the state of Orissa alone, forming 10.8% of the tribal population of India.

The autochthonous tribal populations of India live in virtually inaccessible forests and hills completely isolated from the general stream of the society. They have evolved their own morphogenetical characteristics in their respective ecological niches. Tribal communities in Orissa state are highly vulnerable to hereditary diseases and have a high degree of malnutrition, morbidity and mortality. Tribal health is further compounded by poverty, illiteracy, ignorance of causes of diseases, hostile environment, poor sanitation, lack of safe drinking water, exploitation by elites, faith in traditional beliefs, etc.

Of the hereditary disorders of blood, hemoglobinopathies, glucose-6-phosphate dehydrogenase (G-6-PD) enzyme deficiency and blood group serology are the most important genetic and public health problems in the state of Orissa. Although a large number of genetic studies have been carried out elsewhere among the tribes in India (Roy-choudhury, 1983; Balgir and Sharma, 1988; Bhatia and Rao, 1991; Bhasin et al., 1994; Bhasin and Walter, 2001), genetic data on populations of Orissa especially its indigenous tribal people are limited. Most of the earlier studies were restricted to either a single tribe or a few genetic markers and were mainly descriptive rather than analytical in nature. Further, the previous studies were either hospital based (Kar, 1991) or without following any systematic sampling procedure (Papiha et al., 1988). Hence the exact magnitude of hemoglobinopathies and allied disorders in the state is lacking. ABO blood groups are the most extensively studied genetic system in Orissa

(Papiha et al., 1988; Das et al., 1996; Balgir, 2000), but data on its tribal populations pertaining to even this common genetic marker are scanty as yet.

In view of limited amount of data available on the scheduled tribes vis-à-vis huge reservoir of tribal population in the Orissa state, a genetic investigation has been carried out among as many as 15 major tribal groups inhabiting this central-eastern Indian state.

MATERIALS AND METHODS

Out of the 62 scheduled tribes in the state of Orissa, 15 tribes each comprising more than 1 lakh individuals as per 1991 census were studied. These major scheduled tribes included Bathudi, Bhumiz, Kolha, Lodha and Santal from Mayurbhanj district, Bhuyan, Kharia, Kissan, Munda and Oraon from Sundargarh district, Bhatra from Nawarangpur district, Gond from Kalahandi district, Kondh from Phulbani district, Paraja from Koraput district and Saora from Ganjam and Gajapati districts in the central-eastern part of India.

The tribes in India are by no mean homogeneous in their history, language, culture or social organization. It may be mentioned here that the major scheduled tribes of Orissa belong to three linguistic groups, namely, Indo-Aryan or Indo-Europeans, i.e. Non-Australoid, Austro-Asiatic (Mundari) speakers, i.e. Proto-Australoid, and Dravidian (Gondi or Kuvi) speakers, i.e. Australoid. Proto-Australoid racial group includes Bhumiz, Gadaba, Juang, Kharia, Koda, Kolha, Mahali, Mirdha, Munda, Santal and Saora tribes. Tribes like Bathudi, Bhatra, Binjhal, Bhuyan, Lodha and Saunti belong to non-Australoid racial stock while Australoid racial stock is represented by Gond, Kondh, Kissan, Oraon, Paraja and Pentia Halva tribes.

The highest concentration districts were first identified for each tribe and then the Ashram schools were listed in that locality, of which 4-5 at random selected, representing different

geographical locations in each district. A total of 1959 blood samples were collected from eight districts of the state (Fig.1) after taking informed consent from each individual during the period 1995-2000. It was also ensured to collect blood samples only from unrelated individuals belonging to either sex.

Intravenous blood samples were collected from each student (2-3 ml) under aseptic conditions in EDTA coated vials. The ABO and Rhesus (D) blood group typings were done in the field by slide method, following the instructions of the manufacturer (Tulip Diagnostics, Goa) and the rest of the samples were transported under ice-cold conditions to the laboratory at Bhubaneswar within 24 hours of collection. The sickling test was performed by wet sealed method, using 2% freshly prepared sodium metabisulphite solution following the methodology of Daland and Castle (1948).

Hemoglobin (Hb) electrophoresis was performed in starch-agarose gel in alkaline buffer at pH 8.6 following Dash (1978). Fraction of hemoglobin A₂ was estimated by elution method using cellulose acetate membrane (CAM) in Tris-EDTA-Borate (TEB) buffer at pH 8.9 (Weatherall, 1983; Dacie and Lewis, 1991) and that of fetal hemoglobin as per Weatherall (1983).

The hemoglobin A₂ value of more than 3.5% was taken as the cut off point for beta-thalassemia trait. Glucose-6-phosphate dehydrogenase deficiency was detected by using Dichlorophenol Indophenol (DCIP) dye as described by Bernstein (1962) and subsequently confirmed by procedures of WHO (1967) and Beutler et al. (1979). Allele frequencies were calculated following the standard methods (Mourant et al., 1976).

RESULTS

It is apparent from Table 1 that the distribution of sickle cell disorders varied from zero to 22.4% among 15 major tribes studied here from Orissa. High frequency of the disorders was observed among the Gond (22.4%), Bhatra (18.1%), Paraja (14.8%), Kharia (7.4%) and Saora (7.3%) tribes. While Bhuyan, Kissan, Kolha, Lodha and Oraon tribes lacked them, the distribution of beta-thalassemia showed wide range of variation, i.e. from zero to 8.5% among them, high incidence of the trait was noted among Paraja (8.5%), Santal (8.0%), Lodha (6.7%), Bhatra (6.6%), Kondh (6.3%) and Saora (6.2%). However, no case of sickle cell and beta-thalassemia was detected in the present study.

The distribution of G-6-PD deficiency among

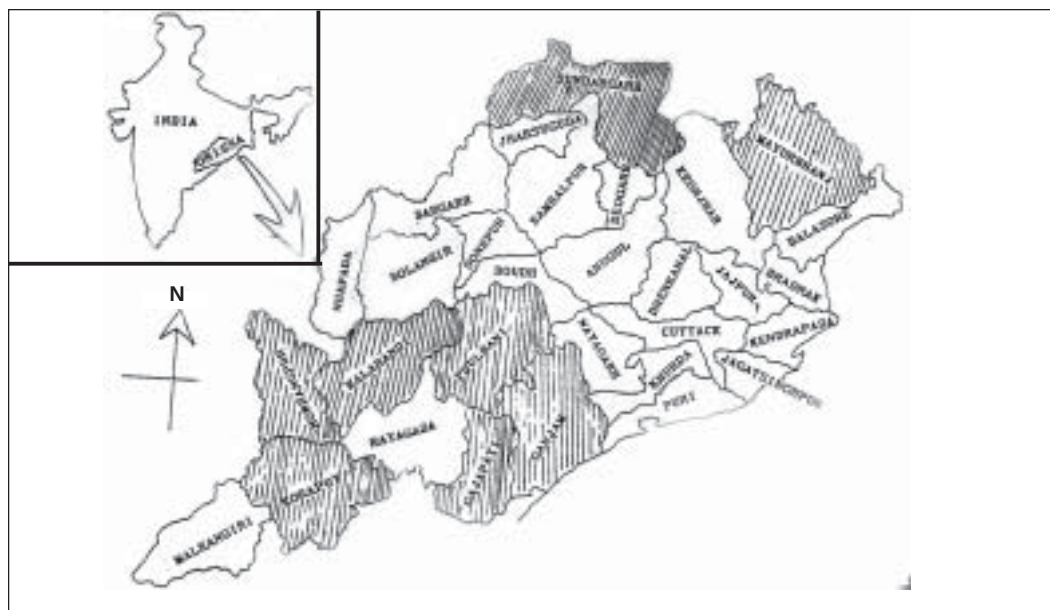


Fig. 1. District map of Orissa state showing study areas (shaded). Inset map of India

Table 1: Distribution of sickle cell disorders, and beta-thalassemia in 15 major Scheduled Tribes of Orissa

Tribe	n	Sickle cell disorders				Beta-Thalassemia			
		Trait	Disease	Total					
Bathudi	95	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)				
Bhatra	166	25 (15.1)	5 (3.0)	30 (18.1)	11 (6.6)				
Bhumiz	116	1 (0.9)	0 (0.0)	1 (0.9)	2 (1.7)				
Bhuyan	92	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				
Gond	219	46 (21.0)	3 (1.4)	49 (22.4)	1 (0.5)				
Kharia	54	4 (7.4)	0 (0.0)	4 (7.4)	1 (1.9)				
Kissan	130	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)				
Kolha	102	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)				
Kondh	254	8 (3.1)	0 (0.0)	8 (3.2)	16 (6.3)				
Lodha	78	0 (0.0)	0 (0.0)	0 (0.0)	6 (6.7)				
Munda	96	3 (3.1)	0 (0.0)	3 (3.1)	5 (5.2)				
Oraon	104	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.9)				
Paraja	176	23 (13.1)	3 (1.7)	26 (14.8)	15 (8.5)				
Santal	100	1 (1.0)	0 (0.0)	1 (1.0)	8 (8.0)				
Saora	177	13 (7.3)	0 (0.0)	13 (7.3)	11 (6.2)				
Total	1959	125 (6.3)	11 (0.6)	136 (6.9)	75 (3.8)				

Figures in parentheses are percentages.

Table 2: Distribution of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in 15 major Scheduled Tribes of Orissa

Tribe	n	Males				Females				Heterozygote	Homozygote
		Deficient	Normal			Deficient	Normal				
Bathudi	95	9 (9.5)	45 (91.8)	4 (8.2)	41 (89.1)	4 (8.2)	41 (89.1)	4 (8.2)	41 (89.1)	4	1
Bhatra	166	11 (6.6)	67 (95.7)	3 (4.3)	88 (91.7)	3 (4.3)	88 (91.7)	3 (4.3)	88 (91.7)	4	4
Bhumiz	116	11 (9.5)	65 (87.8)	9 (12.2)	40 (95.2)	9 (12.2)	40 (95.2)	9 (12.2)	40 (95.2)	1	1
Bhuyan	92	7 (12.9)	25 (83.3)	5 (16.7)	60 (96.8)	5 (16.7)	60 (96.8)	5 (16.7)	60 (96.8)	2	0
Gond	219	13 (5.9)	129 (91.5)	12 (8.5)	77 (98.7)	12 (8.5)	77 (98.7)	12 (8.5)	77 (98.7)	1	0
Kharia	54	5 (14.2)	7 (87.5)	1 (12.5)	42 (91.3)	1 (12.5)	42 (91.3)	1 (12.5)	42 (91.3)	1	3
Kissan	130	5 (5.1)	52 (94.5)	3 (5.5)	73 (97.3)	3 (5.5)	73 (97.3)	3 (5.5)	73 (97.3)	1	1
Kolha	102	10 (9.8)	75 (89.3)	9 (10.7)	17 (94.4)	9 (10.7)	17 (94.4)	9 (10.7)	17 (94.4)	1	0
Kondh	254	17 (6.7)	166 (93.8)	11 (6.2)	71 (92.2)	11 (6.2)	71 (92.2)	11 (6.2)	71 (92.2)	5	1
Lodha	78	4 (5.1)	54 (94.7)	3 (5.3)	20 (95.2)	3 (5.3)	20 (95.2)	3 (5.3)	20 (95.2)	0	1
Munda	96	14 (15.9)	58 (84.1)	11 (15.9)	24 (88.9)	11 (15.9)	24 (88.9)	11 (15.9)	24 (88.9)	3	0
Oraon	104	6 (8.2)	53 (91.4)	5 (8.6)	45 (97.8)	5 (8.6)	45 (97.8)	5 (8.6)	45 (97.8)	1	0
Paraja	176	28 (15.9)	100 (82.6)	21 (17.4)	48 (87.3)	21 (17.4)	48 (87.3)	21 (17.4)	48 (87.3)	4	3
Santal	100	9 (9.0)	72 (92.3)	6 (7.7)	19 (86.4)	6 (7.7)	19 (86.4)	6 (7.7)	19 (86.4)	3	0
Saora	177	14 (7.9)	144 (91.1)	14 (8.9)	19 (100.0)	14 (8.9)	19 (100.0)	14 (8.9)	19 (100.0)	0	0
Total	1959	163 (8.3)	1112 (90.5)	117 (9.5)	684 (93.7)	117 (9.5)	684 (93.7)	117 (9.5)	684 (93.7)	31	15

Figures in parentheses are percentages

15 major scheduled tribes of Orissa is presented in Table 2. The enzyme deficiency was quite high among them, varying from 5.1 to 15.9%. The frequency of this deficiency was high in males (range 4.3-17.4%) than in females (range 0.0-13.6%). Both deficient female heterozygotes and homozygotes were encountered. High incidence of G-6-PD deficiency was observed among Munda (15.9%), Paraja (15.9%), Kharia (14.2%), Bhuyan (12.9%), Kolha (9.8%), Bathudi (9.5%),

Bhumiz (9.5%), Santal (9.0%) and Oraon (8.2%) tribes.

Table 3 shows the distribution of the ABO and Rhesus (D) blood groups among major tribes of Orissa. The frequency of blood group B showed preponderance over blood group A among Bathudi, Bhuyan, Kissan, Kolha, Kondh, Munda, Oraon, Paraja, Santal and Saora tribes. In Bhumiz, Gond, Kharia and Lodha tribes the opposite was true, thereby showing their ethnic

diversity. The frequency of Rhesus-negative phenotype was very low (range zero to 2.1%) among all the tribes of Orissa, and among Bhumiz, Kharia, Kissan, Kolha, Lodha, Munda, Paraja, Santal and Saora tribes it was completely lacking.

Allele frequencies of the various genetic systems studied here are presented in Table 4. It shows that the frequency of sickle cell allele (β^S) varied between zero and 11.9% among the major tribes of Orissa. Beta-thalassemia allele (β^+) was not so very common (range zero to 4.2%) among them. Highest frequency of G-6-PD deficiency allele (Gd^-) was observed among the Paraja

(13.4%) and the lowest in Kissan (2.9%). In the ABO blood groups system, the most commonly observed allele was O, followed by B and A, while in the Rhesus (D) system, allele d varied from zero to 14.5% among the major tribes of Orissa.

DISCUSSION

The preponderance of the allele A of the ABO blood groups over allele B has been observed among Bhumiz, Gadaba, Kharia and Saora (Proto-Australoid tribes), Gond, Kondh and

Table 3: Distribution of the ABO and Rhesus (D) blood groups among 15 major scheduled tribes of Orissa

Tribe	ABO Blood Groups					Rhesus Blood Groups	
	<i>n</i>	A	B	AB	O	Negative	Positive
Bathudi	95	29 (30.6)	34 (35.8)	14 (14.7)	18 (18.9)	2 (2.1)	93 (97.9)
Bhatra	166	42 (25.3)	42 (25.3)	16 (9.6)	66 (39.8)	1 (0.6)	165 (99.4)
Bhumiz	116	40 (34.5)	30 (25.9)	10 (8.6)	36 (31.0)	0 (0.0)	116 (100.0)
Bhuyan	92	26 (28.3)	32 (34.8)	6 (6.5)	28 (30.4)	1 (1.1)	91 (98.9)
Gond	219	73 (33.3)	47 (21.5)	15 (6.8)	84 (38.4)	1 (0.5)	218 (99.5)
Kharia	54	20 (37.0)	18 (33.3)	5 (9.3)	11 (20.4)	0 (0.0)	54 (100.0)
Kissan	130	36 (27.7)	39 (30.0)	13 (10.0)	42 (32.3)	0 (0.0)	130 (100.0)
Kolha	102	30 (29.4)	36 (35.3)	6 (5.9)	30 (29.4)	0 (0.0)	102 (100.0)
Kondh	254	75 (29.5)	94 (37.0)	27 (10.6)	58 (22.8)	1 (0.4)	253 (99.6)
Lodha	78	34 (43.6)	25 (32.0)	12 (15.4)	7 (9.0)	0 (0.0)	78 (100.0)
Munda	96	31 (32.3)	34 (35.4)	10 (10.4)	21 (21.9)	0 (0.0)	96 (100.0)
Oraon	104	30 (28.9)	38 (36.5)	13 (12.5)	23 (22.1)	1 (1.0)	103 (99.0)
Paraja	176	49 (27.8)	59 (33.5)	17 (9.7)	51 (29.0)	0 (0.0)	176 (100.0)
Santal	100	20 (20.0)	39 (39.0)	9 (9.0)	32 (32.0)	0 (0.0)	100 (100.0)
Saora	177	36 (20.3)	64 (36.2)	27 (15.3)	50 (28.3)	0 (0.0)	177 (100.0)
Total	1959	571 (29.1)	631 (32.2)	200 (10.2)	557 (28.5)	7 (0.4)	1952 (99.6)

Figures in parentheses are percentages

Table 4: Allele frequencies in 15 major Scheduled Tribes of Orissa

Tribe	β^A	β^S	β^+	Gd^-	A	B	O	d	D
Bathudi	0.995	0.005	0.000	0.071	0.267	0.303	0.430	0.145	0.955
Bhatra	0.895	0.105	0.033	0.057	0.301	0.301	0.398	0.077	0.923
Bhumiz	0.996	0.004	0.009	0.076	0.252	0.197	0.551	0.000	1.000
Bhuyan	1.000	0.000	0.000	0.045	0.212	0.253	0.535	0.105	0.895
Gond	0.881	0.119	0.003	0.044	0.227	0.154	0.619	0.071	0.929
Kharia	0.963	0.037	0.009	0.080	0.300	0.275	0.425	0.000	1.000
Kissan	1.000	0.000	0.007	0.029	0.207	0.222	0.571	0.000	1.000
Kolha	1.000	0.000	0.010	0.125	0.220	0.258	0.522	0.000	1.000
Kondh	0.984	0.016	0.031	0.054	0.243	0.293	0.464	0.063	0.937
Lodha	1.000	0.000	0.033	0.051	0.411	0.328	0.261	0.000	1.000
Munda	0.984	0.016	0.026	0.114	0.265	0.285	0.450	0.000	1.000
Oraon	1.000	0.000	0.009	0.040	0.242	0.293	0.467	0.100	0.900
Paraja	0.917	0.083	0.042	0.134	0.214	0.251	0.535	0.000	1.000
Santal	0.995	0.005	0.040	0.074	0.156	0.277	0.567	0.000	1.000
Saora	0.963	0.037	0.031	0.071	0.168	0.276	0.556	0.000	1.000

β^A = Hb A allele; β^+ = Beta-thalassemia allele; β^S = Sickle cell allele

Gd^- = G-6-PD deficiency allele; d = Rhesus negative allele; D = Rhesus positive allele

Paraja (Australoid tribes), Bhatra, Lodha (nonAustraloid tribes) which is a characteristic feature of Mongoloid populations in the North-Eastern part of India (Roychoudhury, 1983). Similarly, the allelic frequency of Rhesus blood group allele d is low among the Mongoloid populations of North-Eastern India (Balgir, 1992) and almost all the major tribes of Orissa have shown either a reduced frequency of this allele or complete absence of the allele among several of them like Bhumiz, Kharia, Kissan, Kolha, Lodha, Munda, Paraja, Pertia Halva and Saora. This suggests admixture among major tribes of Orissa with that of Mongoloid elements of North-Eastern India.

The distribution of the X-linked enzyme G-6-PD deficiency among the major tribal populations of Orissa is interesting one. This enzyme deficiency is the most prevalent clinically significant disorder in man and is one of the etiological factors associated with severe neonatal jaundice in full-term normal weight infants in India (Balgir, 1989). There is a high degree of variation in the frequency of G-6-PD deficiency in the major tribes of Orissa. High frequencies of this enzyme deficiency have been observed elsewhere among Mongoloid tribal populations like Angami Naga (27.0%), Adi (19.4%), Apatani (16.7%), Nishi (16.0%), Rabha (15.8%), Mikir (15.6%) (Seth and Seth, 1971; Das et al., 1982; Balgir, 1989, 1995) in North-Eastern India and Warli (17.0%), Parsis (15.7%), Madia (14.4%) in Western India (Baxi et al., 1963; Kate, 1979). The high frequency of G-6-PD deficiency observed among the major tribes of Orissa in this study, especially among Bathudi, Bhuyan, Juang, Kolha, Munda, Paraja and Santal further testifies the contention of diffusion and gene flow of Mongoloid elements among the contemporary tribal populations of Orissa. It is interesting to note that the deficiency was equally high among the Austro-Asiatic as well as Indo-Aryan speakers. The most likely explanation for this situation is that the Austro-Asiatic speakers whose ancestors probably came to Central India or Southern Orissa during prehistoric times and brought this deficient allele with them and later, through the process of admixture, spread it into the populations speaking Dravidian and Indo-Aryan languages. That is why the G-6-PD deficiency is low (2.9-5.4%) among the Dravidian tribes of Orissa, viz. Gond, Kondh, Kissan and Oraon.

Further, Orissa is highly endemic for malaria, especially of type *Plasmodium falciparum* which takes a high toll of lives in the state every year. The individuals with G-6-PD deficiency have an advantage over the non-deficient persons in that the malarial parasites do not survive longer in these individuals and die due to lack of favourable bio-environment. Hence, the enzyme deficient individuals in malarial endemic bio-environment do not suffer severely from malaria rather they have adapted themselves to this kind of bioenvironment and have developed to some extent immunity against it.

Recently, the G-6-PD enzyme deficiency among the tribes (including the tribes of the present study like Bathudi, Bhuyan, Munda and Santal) of India has been identified as a new variant, called "G-6-PD Orissa" which has only 10-20% of the normal enzyme activity and normal electrophoretic mobility, but has five-fold higher Michael's constant for the substrates which actually translates roughly into five-fold lower activity at limiting substrate concentrations and shows the increased thermostability than normal enzyme (Kaeda et al., 1995). This means that the anti-malarial drugs like primaquine and many other compounds such as phenacetin, furadantin, certain sulphonamides and acetyl salicylic acid (aspirin), etc. should be administered with caution among the tribal populations of India including of Orissa, which may cause hemolytic crisis and, sometimes may even be fatal.

In the present study, the frequency of sickle cell allele was comparatively high in the Gond (11.9%) and Bhatra (10.5%). The latter tribe is Indo-Aryan speaker and the former is a Dravidian tribe. Also, the allele was found absent among the Bhuyan, Kissan, Kolha, Lodha and Oraon tribes in the present study. This shows that the sickle cell trait which was probably prevalent initially only in the Dravidian populations has later penetrated the Indo-Aryan populations or vice versa, and this indicates the genetic admixture among the present tribal populations. Further support to such a hypothesis has come from studies of origin of sickle cell gene in India (Balgir, 2001).

It is interesting that the distributions of sickle cell (β^S) and beta-thalassemia (β^+) alleles showed that, when the former is depressed, the latter is elevated (Tables 1 and 4). Whether the frequency clines of these two alleles represent gene flow across the region or provide a compensatory

selective mechanism against malaria, is worth exploring. The findings of the present study suggest gene flow across the tribal populations as well as the selective mechanism against malaria which is rampant among them.

Both linguistic and racial admixtures have occurred among the major tribes of Orissa who manifest genetic heterogeneity and diversity with respect to Mongoloid, Australoid, Proto-Australoid and Non-Australoid characteristics in the state.

Further, although some of the variations may be due to the sample size, number, and geographic distance between tribal populations, variables tested and breeding structure of the population, the present analysis strongly suggests that the intrastructure of these tribal populations is highly influenced by the local inbreeding (endogamy) within each tribal population. These findings are further supported by the earlier studies of Das et al. (1996) and Balgir (2002).

CONCLUSIONS

Tribal communities constitute an important segment of the society in India. They are highly vulnerable to hereditary diseases and have a high degree of morbidity and mortality affecting their general health in Orissa. Major tribes of Orissa studied here have shown heterogeneity and diversity with respect to various genetic markers. This may be due to continuous pressures of gene diffusion from different waves of people penetrating their territory and habitats in Orissa in course of invasions and migrations from the North-Western (Indo-Aryan) and North-Eastern (Mongoloid) parts of India during historical and ancient times. In addition to inter-tribal admixture, and sometimes intra-tribal isolation have also occurred as reflected by the genetic parameters studied here. There is a strong evidence for the operation of a compensatory selective mechanism against malaria by way of high prevalence of the sickle cell, beta-thalassemia and G-6-PD deficiency among different major tribes of Orissa.

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KEYWORDS Blood Groups. Sickle Cell Disorders. Beta-thalassemia. G-6-PD Deficiency. Tribal Populations. Genetic Heterogeneity. Orissa

ABSTRACT The autochthonous tribes of India constitute an important segment of the society. Genetic data about the tribal people in the state of Orissa are limited. The present investigation deals with the distribution of five genetic markers, namely, ABO and Rhesus (D) blood groups, sickle cell disorders, beta-thalassemia trait, and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency among fifteen major scheduled tribes scattered in eight different districts of Orissa. A preponderance of blood group B over A, and a low incidence (0-2.1%) of Rhesus-negatives among most of them have been observed. The deficiency of G-6-PD enzyme was quite high, varying from 5.1 to 15.9%; both the deficient female heterozygotes and homozygotes were encountered. The frequency of sickle cell allele varied between zero and 21.5% in the present study, but was comparatively higher in Paraja (21.5%), Gond (11.9%) and Bhatra (10.5%) tribes. Genetic diversity and heterogeneity with respect to the genetic markers studied in the major scheduled tribes of Orissa was evident and this indicated not only inter-tribal admixture but also the diffusion with other racial groups of India. These results have been discussed in the light of previous studies carried out from the state.

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