HEMATOLOGICAL PROFILE OF TWENTY-NINE TRIBAL COMPOUND CASES OF HEMOGLOBINOPATHIES AND G-6-PD DEFICIENCY IN RURAL ORISSA

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ABSTRACT

***BACKGROUND:** Hematogenetic disorders are commonly encountered in Orissa state in Central-Eastern India. Hemoglobinopathies and G-6-PD deficiency are the most frequently occurring hereditary hemolytic disorders causing high morbidity and mortality in vulnerable people. **AIMS:** There is no study available reporting combined condition of hemoglobinopathies and G-6-PD deficiency in a single individual from India. This study aims to assess the coincidence of G-6-PD enzyme deficiency with different hemoglobinopathies and β-thalassemia and to evaluate the influence of combined conditions on the hematological expression. **SETTINGS AND DESIGN:** The study was carried out in rural Orissa with a random sampling procedure. **MATERIALS AND METHODS:** Following the standard methodology and techniques, this study highlights 29 tribal cases of compound occurrence of hemoglobinopathy with G-6-PD deficiency in a randomly conducted study in Sundargarh district of Orissa. **STATISTICAL ANALYSIS:** Results were subjected to statistical analysis. **RESULTS:** Both female heterozygotes and homozygotes of G-6-PD deficiency in association with different hemoglobinopathies showed reduced values of hematological indices: hemoglobin level, MCV, MCH, MCHC and RBC in comparison to normals. Red cell indices were found further reduced in male G-6-PD deficiency concurrence with hemoglobinopathies in homozygous condition, i.e. sickle cell disease (HbSS) or hemoglobin E disease (HbEE). Hematological indices were significantly lower except WBC counts and fetal hemoglobin in male G-6-PD deficiency with co-existing homozygous sickle cell disease in comparison with counterpart sickle cell trait and normal controls. **CONCLUSIONS:** Hemoglobin polymorphism with G-6-PD deficiency is advantageous to the community against lethal effects of malaria especially against Plasmodium falciparum at population level, but their combination is harmful at the individual level because of low levels of red cell indices to cope with the routine human physiology.*

***Key words:** Compound hereditary disorders, G-6-PD deficiency, hemoglobinopathies, hemolytic anemia, Orissa, tribal communities.*

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INTRODUCTION

Inherited hemoglobin disorders are caused by structural abnormalities including abnormal synthesis of chains, and represent serious monogenic disorders in the world. The genes for α-like globin chains are found in a cluster at the tip of the short arm of chromosome 16, while those for β–like chains are found on chromosome 11. The complete nucleotide sequence of these regions has been determined and the molecular pathology of most hemoglobin disorders is well defined.[1] The most commonly occurring structural defect of hemoglobin is sickle cell hemoglobinopathy, prevalent in the tropical and subtropical regions of the world. Hemoglobin E is most common variant in Southeast Asia, where its prevalence is estimated to be around 30%. Sporadic cases of hemoglobin D are also encountered in many parts of the Indian subcontinent. Detrimental thalassemias result from genetic defects that cause reduced synthesis of polypeptide globin chains to form hemoglobin. Clinical manifestations and severity of these structural abnormalities are qualitative in nature, whereas, in thalassemia syndromes these are related to the degree of reduction of α- or β-globin chain synthesis in an individual.

The glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is the most common self-limiting X-linked enzymopathy affecting around 200-400 million people in tropical and subtropical regions of the world. The gene for G-6-PD is located on the long arm of X-chromosome at q28 position. The enzyme is required in red cells for the production of reduced glutathione (GSH) from oxidized glutathione (GSSG) in the hexose monophosphate (HMP) shunt pathway. In the absence of reduced glutathione (GSH), oxidant stress can lead to hemolysis of erythrocytes. A host of agents like antibiotics, antimalarials, analgesics, infections, broad (flat) fava beans (vicia faba) and acute illnesses are associated with hemolysis in G-6-PD deficiency. It plays a critical role in regulation of cell growth and its survival. The deficient fibroblasts suffer growth retardation in humans and premature cellular senescence. Major clinical manifestations are drug induced sudden pallor, jaundice, hemolytic anemia, darkening of urine, neonatal jaundice, neonatal hyperbilirubinemia and chronic non-spherocytic hemolytic anemia. There is no specific cure and treatment for G-6-PD deficiency, and the management varies depending on the type of enzyme deficiency and the nature of drug dosage. The hemolysis is usually self-limiting and is corrected after discontinuation of the drug. The prevalence of G-6-PD deficiency varies from 0 to 27% in different castes, tribes, and ethnic groups, religious and linguistic groups in India.[2] The deficiency was observed varying from 0.4 to 17.4% in the tribal communities of Orissa.[3] Most of the individuals remain undiagnosed due to lack of awareness and non-availability of testing facilities in India.[4]

These disorders are genetically independent, and assort independently. Both hereditary hemolytic disorders are prevalent in Central-Eastern India. It was estimated that on an average 19.32% of the people in Orissa are victims of hemoglobinopathies, or every fifth person has this condition. Of these, 13.2% suffer from sickle cell disorders (sickle cell trait = 8%; sickle cell disease = 4.0%; sickle cell-β-thalassemia = 1.2%) alone.[5] In tribal populations, the frequency of β-thalassemia and sickle cell disorders varies between 0-10.8% and 0-22.4%, respectively.[3, 6]

The reports regarding the relationship of G-6-PD deficiency with sickle cell disease are conflicting. In areas of prevalence of both these genes, their coincidence has been considered significantly higher by some workers,[7-10] while others have not supported this observation.[11,12] Similarly, controversy has prevailed regarding the beneficial, deleterious or neutral effects of G-6-PD deficiency on the clinical status of sickle cell disease.[9,10,12-17] Although both hemoglobinopathies and G-6-PD deficiency are prevalent in malaria endemic areas in India, to the best of our knowledge, no study has ever reported combined conditions in a single individual from India. The present study highlights 29 tribal cases with compound occurrence of hemoglobinopathies and G-6-PD deficiency in a randomly conducted study in Sundargarh district of northwestern Orissa.

MATERIALS AND METHODS

This study was a part of our larger project carried out for random screening of two major tribal communities, Bhuyan and Kharia, for G-6-PD deficiency and hemoglobin variants in Sundargarh district of northwestern Orissa between July 2000 and September 2004. Ethical clearance from Ethical Committee of Regional Medical Research Centre, Bhubaneswar was obtained prior to conducting the study and informed consent was taken before taking the blood sample from patients/controls. A total of 836 Bhuyan (407 males and 429 females) and 767 Kharia (377 males and 390 females) tribals belonging to all age groups were screened. From this screening, a total of 29 cases of different hemoglobinopathies with G-6-PD deficiency reported in the present study were encountered.

Blood collection

About 2-3 mL intravenous blood samples were collected using ethylene diamine tetra acetic acid (EDTA) as anticoagulant with disposable syringes and needles from each individual after obtaining the informed/written consent in the presence of a doctor and community leaders. Any other ailment present was treated/referred to local health facilities. Blood samples so collected were transported to a laboratory at Bhubaneswar under ice-cold conditions within 24 hours of collection. Laboratory investigations were carried out following the standard procedures after cross checking for quality control from time to time. Hematological parameters were studied using an automated Blood Cell Counter (Model - MS4, Melet Schloesing Laboratories, Cergy-Pontoise Cedex, France).

Laboratory analysis

The sickling test was performed on red cells for all the blood samples using freshly prepared sodium metabisulphite solution as reducing agent[18] to determine the presence or absence of sickle hemoglobin. The routine hemoglobin electrophoresis was carried out on cellulose acetate membrane (CAM) in Tris-EDTA-Borate buffer at pH 8.9 and quantification of A_2 fraction of hemoglobin by elution method.[19] A value of more than 3.5% of A_2 fraction of adult hemoglobin was taken as cut-off point for determining β-thalassemia trait. Estimation of fetal hemoglobin was done following the procedures of Betke and coworkers as modifications described by Weatherall.[19] Confirmation for the presence of hemoglobin D or E was done by performing

the citrate agar gel electrophoresis at pH 6.2, and hereditary persistence of fetal hemoglobin (HPFH) was done respectively as described elsewhere.[20-22] However, the data presented here refer only to probands. Hemoglobin variant (made for Bio-Rad Diagnostics Group, Hercules California, USA) analysis was carried out to confirm doubtful cases. Family studies were carried out to confirm the diagnosis of probands only, wherever it was felt necessary.

The G-6-PD enzyme deficiency was primarily detected by using dichlorophenol indophenol (DCIP) dye as described by Bernstein.[23] Females heterozygous for G-6-PD deficiency have two populations of cells, one with normal G-6-PD activity and the other deficient. This is the result of inactivation (Lyon's hypothesis) of one of the two X chromosomes in individual cells early in the development of the embryo. All progeny (somatic) cells in females will have the characteristics of only the active X chromosome. The total G-6-PD activity of blood in female will depend on the proportion of normal to deficient cells. In most cases, the activity will be between 20 and 80% of the normal. However, a few heterozygotes (about 1%) may have almost only normal or almost only G-6-PD deficient cells. The present study has not encountered any such ambiguity; therefore, there were either 60-80% of the cells normal or deficient in all cases. Subsequent confirmation was done by following the Beutler *et al.*[24] and WHO procedures[25] if any doubt arose on the detection of G-6-PD deficiency.

RESULTS

In the present study, out of 29 cases of G-6-PD deficiency with concurrent occurrence of different hemoglobinopathies, 12 (9 males and 3 females) belonged to Bhuyan tribal community and 17 (12 males and 5 females) were of Kharia tribe from Sundargarh district of Orissa. It is apparent from Table 1 that the mean values of normal cases without G-6-PD deficiency/hemoglobinopathies for most of the hematological indices, in general, are lower than the standards in the two major scheduled tribes of Orissa. This may be due to the prevalence of iron deficiency anemia, folate deficiency, vitamin B_{12} deficiency, parasitic (malaria) infection, parasitic infestations, hepatic (liver) disease, malignancy of bone marrow or wild food-induced toxemia in these two tribal communities. Since the undertaken study was primarily focused on hemoglobinopathies, the other aspects of hemolytic anemia in details in these subjects were not studied.

It was observed that both heterozygote and homozygote females with G-6-PD deficiency with co-existence of different hemoglobinopathies, i.e. Hb D, Hb E, Hb S showed reduced values of almost all hematological indices especially the level of hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell count (RBC) in comparison with the normal controls. The red cell indices were found further reduced in male G-6-PD deficients with co-existing hemoglobinopathies in homozygous condition, i.e. sickle cell disease (HbSS) or hemoglobin E disease (HbEE) [Table 1]. However, the red cell indices picture was variable in males with G-6-PD deficiency with co-existing heterozygous (carrier) hemoglobinopathies, i.e. sickle cell trait (Hb AS), hemoglobin D trait (Hb AD),

Table 1: Tribal distribution with different hemoglobinopathies and G-6-PD deficiency and their hematological indices

Tribe	*Hb-pathies/ G6PD*	*Age*	*Sex*	*Hb (g/di)*	*RBC (x 10^6/µl)*	*MCV (fl)*	*HCT (%)*	*MCH (pg)*	*MCHC (g/dl)*	*WBC (x 10^6/µl)*	*HbA_2 (%)*	*HbF (%)*	*HbS/D (%)*	*Blood Groups*
Paik Bhuyan	β-Thal/Def.	80	M	9.6	4.06	80.5	32.7	23.6	29.3	3.44	5.1	0.4	-	O+
Dudh Kharia	β-Thal/Def.	58	M	7.6	3.31	79.9	26.4	22.1	27.6	9.62	6.6	0.4	-	AB+
Paudi Bhuyan	β-Thal/Def.	25	M	11.3	4.90	84.2	41.1	23.1	27.4	6.10	6.3	0.6	-	B+
Paraja Bhuyan	β-Thal/Def.	15	M	12.9	5.16	84.9	43.8	25.0	29.5	5.56	4.5	0.4	-	A+
Paraja Bhuyan	β-Thal/Def.	7	M	10.3	4.84	78.2	37.8	21.3	27.2	4.98	5.1	0.8	-	A+
Paraja Bhuyan	β-Thal/Def.	6	M	11.0	5.08	72.3	36.7	21.7	30.0	4.85	5.0	0.4	-	O+
Mean	β-Thal/Def.	30.8	M	10.45	4.56	80.0	36.42	22.8	28.5	5.76	5.43	0.5	-	-
SD		±30.4		±1.78	±0.72	±4.57	±6.20	±1.37	±1.23	±2.09	±0.82	±0.17	-	-
Dhelki Kharia	β-Thal/Def. Homo.	55	F	10.7	4.94	80.0	39.6	21.7	27.0	6.81	4.4	0.5	-	O+
Dudh Kharia	β-Thal/Def. Hetero.	30	F	11.4	3.89	73.3	28.5	23.9	32.7	3.61	4.3	2.4	-	B+
Paraja Bhuyan	β-Thal/Def. Hetero.	17	F	10.3	4.37	79.2	34.6	23.6	29.8	5.83	5.2	0.6	-	B+
Dhelki Kharia	SS/Def.	22	M	10.2	5.78	65.8	38.1	17.7	26.9	8.99	2.0	13.7	84.3	A+
Dhelki Kharia	AS/Def.	41	M	8.8	3.79	76.0	33.9	16.5	24.5	5.42	2.1	1.9	17.1	A+
Paraja Bhuyan	AS/Def.	40	M	14.3	5.49	69.8	38.3	26.0	37.3	8.21	1.9	0.7	13.4	O+
Dhelki Kharia	AS/Def.	25	M	13.9	6.53	73.8	48.2	21.2	28.8	4.78	3.2	0.5	12.1	B+
Paik Bhuyan	AS/Def.	22	M	13.6	6.29	80.6	48.7	21.6	26.8	8.45	1.8	0.7	13.3	A+
Dhelki Kharia	AS/Def.	14	M	10.8	5.94	71.0	42.2	18.1	25.5	3.97	2.2	1.1	24.0	B+
Dhelki Kharia	AS/Def.	11	M	10.9	5.47	75.4	41.2	20.0	26.5	6.51	2.1	1.5	23.5	B+
Dhelki Kharia	AS/Def.	8	M	13.0	7.56	74.2	48.0	19.8	26.7	7.44	1.0	0.6	25.5	A+
Dhelki Kharia	AS/Def.	6	M	11.5	5.93	74.5	44.2	19.4	26.0	6.34	3.1	0.5	25.7	O+
Paik Bhuyan	AS/Def.	5	M	11.1	4.61	75.6	38.4	24.1	28.9	8.60	2.7	0.3	24.1	B+
Dhelki Kharia	AS/Def.	2	M	8.8	4.29	68.7	29.4	20.5	29.9	7.39	3.0	2.7	30.7	AB+
Mean	AS/Def.	17.4	M	11.67	5.59	73.96	41.25	20.72	28.09	6.71	2.31	1.05	20.94	-
SD		±14.2		±1.98	±1.13	±3.45	±6.4	±2.75	±3.64	±1.59	±0.69	±0.76	±6.43	-
Dhelki Kharia	AS/Def.Hetero	70	F	10.4	6.30	70.2	44.2	19.6	27.9	6.47	2.3	0.9	20.3	A+
Paraja Bhuyan	AS/Def.Hetero.	57	F	9.5	4.97	68.2	33.8	19.1	28.1	6.28	1.7	0.8	12.9	B+
Dhelki Kharia	AS/Def.Homo.	5	F	6.8	5.05	51.2	25.8	13.4	26.3	7.65	2.8	0.6	24.8	B+
Dhelki Kharia	AS/Def.Homo.	4	F	10.1	4.43	73.0	32.3	22.7	31.2	7.24	2.0	2.2	23.7	B+
Paik Bhuyan	AD/Def.	60	M	10.2	4.34	82.4	35.7	23.5	28.5	4.32	1.1	0.5	30.7	O+

Table 1: Continued ....

Tribe	Hb-pathies/ G6PD	Age	Sex	Hb (g/di)	RBC (x 10^6/μl)	MCV (fl)	HCT (%)	MCH (pg)	MCHC (g/dl)	WBC (x 10^6/μl)	HbA_2 (%)	HbF (%)	HbS/D (%)	Blood Groups
Paik Bhuyan Dhelki	AD/Def.Hetero.	38	F	11.3	4.89	84.6	41.3	23.1	27.3	6.99	1.3	0.3	28.7	B+
Kharia Dhelki	EE/Def.	45	M	9.1	5.23	65.0	34.0	17.4	26.7	6.41	98.2	1.8	-	A+
Kharia Dhelki	AE/Def.	60	M	10.2	5.13	65.3	33.4	18.9	26.7	7.18	28.1	0.8	-	AB+
Kharia Dhelki	AE/Def.	5	M	8.6	4.67	65.7	30.6	18.4	28.1	9.62	28.0	0.4	-	A+
Normal (N=1574)				11.0	5.1	77.1	39.3	21.6	28.0	6.2	2.3	1.4	-	-
SD				±1.6	±0.7	±7.8	±5.4	±2.9	±2.2	±2.4	±0.5	± 0.6	-	-

β-Thal.= β-Thalassemia Trait; Def.= G-6-PD deficiency; Homo.= Homozygote; Hetero.= Heterozygote; SS = Sickle Cell Anemia; AS = Sickle Cell Trait; AD = Hemoglobin D Trait; EE = Hemoglobin E disease; AE = Hemoglobin E Trait

hemoglobin E trait (Hb AE), and β-thalassemia trait in comparison to normal controls. However, some cases did show normal or identical indices to the normal controls, whereas the others showed reduced values under natural (field) environmental conditions [Table 1]. The mean values of hematological indices were slightly better in sickle cell trait (Hb AS), and hemoglobin D trait (Hb AD) cases than in the β-thalassemia carriers of G-6-PD deficiency.

The mean hematological indices in male G-6-PD deficients with co-existing β-thalassemia trait, the level of hemoglobin, RBC counts, HCT, WBC counts and fetal hemoglobin although statistically not significant, were lower than in the normal controls. These indices were found further reduced in both heterozygous and homozygous female G-6-PD deficients with co-existing β-thalassemia trait, showing concomitant negative effect of these two hereditary diseases. It was further noticed that this effect of lowering the red cell indices was milder in males than in females [Table 1].

The mean values of MCV, MCH and fetal hemoglobin were lower and those of hemoglobin level, RBC counts, HCT, MCHC and WBC counts, higher in male G-6-PD deficients with co-existing sickle cell trait in comparison with normal controls. The values of hematological indices were much lower in both heterozygous and homozygous female G-6-PD deficients with concurrent sickle cell trait in comparison with counterpart males and normal controls [Table 1]. The interaction effect of G-6-PD deficiency with sickle cell gene (trait) in lowering the red cell indices was more marked in females than in males. However, almost all the hematological indices were significantly lower except WBC counts and fetal hemoglobin level in male G-6-PD deficiency with co-existing homozygous sickle cell disease in comparison with counterpart sickle cell trait and normal controls.

A similar picture emerged out of the interaction of G-6-PD deficiency with conjoint occurrence of hemoglobin D or E [Table 1] in individuals of the two tribal communities of Orissa. Distribution of ABO and Rhesus (D) blood groups in individual cases is presented in Table 1 as an additional parameter of hemolytic anemia, although it was not directly related to the present study.

DISCUSSION

It is a rare occasion when an individual is afflicted with two independently inherited

hemolytic defects, resulting in severe clinical and hematological manifestations. The possible interaction of one of the hemoglobinopathies, i.e. sickle cell disease and G-6-PD deficiency has been the subject of a number of studies in different populations.[7-10,12,14,15,17,26] The state of Orissa is hyper endemic for malaria and the high frequency of these disorders is related to selective advantage against malaria.[27]

The present study reported 29 cases afflicted with double hereditary hemolytic disorders for the first time in two tribal communities of Sundargarh district in northwestern Orissa. It was observed that the frequency of different hemoglobinopathies and G-6-PD deficiency was very high, being 9.8 and 17.0, and 13.3 and 24.9, respectively in the Bhuyan and Kharia tribal communities.[28,29] Since they are endogamous people practicing clan exogamy and area endogamy, the chances of getting these two defects together in an individual is equally high as tribal endogamy leads to increased homozygosity for recessively inherited disorders in malaria endemic populations. High incidence of G-6-PD deficiency has been reported in areas of the Central Eastern India[28] where the different hemoglobinopathies, i.e. sickle cell disease, hemoglobin E, hemoglobin D, and β-thalassemia genes are also prevalent. The present study, conducted with an aim to assess the coincidence of G-6-PD enzyme deficiency with different hemoglobinopathies and β-thalassemia, and to evaluate the influence of combined hemolytic disorders on the hematological expression, showed detrimental effects of these two conditions on an individual.

It has been observed in the present study that the mean values of MCV, MCH and fetal hemoglobin were lower and those of hemoglobin level, RBC counts, HCT, MCHC and WBC counts, were higher in male sickle cell traits with co-existing G-6-PD deficiency in comparison with normal controls. The values of hematological indices were significantly much lower in female sickle cell traits with concurrent G-6-PD deficiency in both heterozygotes and homozygotes in comparison with counterpart males and normal controls [Table 1]. The effect of interaction of G-6-PD deficiency with sickle cell gene (trait) in lowering the red cell indices was more marked in females than in males. However, almost all the hematological indices were significantly lower except WBC counts and fetal hemoglobin level in male homozygous sickle cell patients with co-existing G-6-PD deficiency in comparison with counterpart sickle cell trait and normal controls. These findings are consistent with those of Bouanga *et al*.[12] in that there is no advantage of the association of G-6-PD deficiency with sickle cell disease. It suggests that sickle cell disease patients with G-6-PD deficiency are prone to increased hemolysis when exposed to drugs or oxidative stress. These findings are contradictory to Bernstein *et al*.[9] in that a young red cell population associated with the sickle cell gene leading to elevated G-6-PD levels in G-6-PD deficient males suggests that sickle hemoglobin may exert a beneficial effect on G-6-PD deficiency, rather than the reverse. These red cells may be better able to deal with oxidative stress, which can precipitate severe hemolytic disease in G-6-PD deficiency. However, Mohammad *et al*.[16] in a study on the coexistence and interaction of sickle cell disease with G-6-PD deficiency in Bahrain found a positive correlation between G-6-PD deficiency and sickle cell disease.

On the other hand, Awamy[10] found increased frequency of interaction of G-6-PD deficiency with sickle cell disease but found no effect of this enzyme defect on the clinical and hematological status of homozygous sickle cell disease in Saudi Arabia. An appraisal of clinical status in homozygous sickle cell disease patients with and without G-6-PD deficiency showed no ameliorating or harmful effect of this enzyme abnormality upon the sickle cell disease. Awamy[10] argued that G-6-PD deficiency neither exacerbated nor mitigated the frequency of painful crisis, incidence of infection or anemic episodes in a cohort study of 83 babies. Further, hematologically, no significant differences were observed in MCHC, MCV, reticulocyte counts and fetal hemoglobin levels in homozygous sickle cell disease patients with and without G-6-PD deficiency,[10] contrary to our findings. Similarly, Diop *et al*.[17] in a study on prevalence and morbidity of G-6-PD deficiency in homozygotes of sickle cell disease in Senegal found no difference between the clinical severity of sickle cell disease with or without G-6-PD deficiency.

Awah and Uzoegwu[26] in a study on the influence of sickle heterozygous status and G-6-PD deficiency on the clinico-hematological profile of *Plasmodium falciparum*-infected children in Nigeria found that inheriting both genetic defects reduces the profligacy of malaria parasite and hence, ameliorates the severity of acute *falciparum* malaria. Consequently, selective advantage against fatal *falciparum* malaria seems to be conferred since malarial anemia, parasitemia and severe malarial symptoms were significantly reduced. It was noted in the present study that both heterozygote and homozygote females with G-6-PD deficiency with co-existence of different hemoglobinopathies, i.e. Hb D, Hb E, Hb S showed reduced values of almost all hematological indices especially the level of hemoglobin, MCV, MCH, MCHC and RBC counts in comparison with the normal controls. The red cell indices were found further reduced in male G-6-PD deficients with co-existing hemoglobinopathies in homozygous condition, i.e. sickle cell disease (HbSS) or hemoglobin E disease (HbEE) [Table 1]. However, the red cell indices picture was variable in males with G-6-PD deficiency with co-existing heterozygous (carrier) hemoglobinopathies, i.e. HbAS, HbAD, HbAE, and β-thalassemia trait in comparison with normal controls.

Further, a trend for inverse relationship between the sickle cell allele and G-6-PD deficiency, and sickle cell and β-thalassemia allele in a cross-section of malaria endemic *(Plasmodium)* tribal communities was emphasized in Orissa.[27] When the frequency of sickle cell allele decreases in a cross-section of malaria endemic tribal population, the frequency of G-6-PD enzyme deficiency and β-thalassemia allele increases and vice versa. The detrimental variant of sickle cell allele is being replaced by G-6-PD deficiency allele because of mild clinical manifestations of enzyme deficiency in comparison with crippling manifestations of sickle cell allele in Orissa. In Sundargarh district alone in Orissa between 25-61 deaths due to malaria have been reported every year. [27] This means natural selection has played a major role in favor of sickle cell, β-thalassemia and G-6-PD mutational alleles so that they had probably evolved as a protective mechanism against the lethal effects of malaria in this part of the country.[27]

The polymorphism of hemoglobin variants and G-6-PD deficiency is advantageous to the community against the lethal effects of hyper malaria especially against infection of *Plasmodium falciparum* at population level, but their combination is harmful at individual level because of low levels of red cell indices to cope with the routine human physiology. These findings get further support from the observation of Buanga *et al*.[12] Moreover, the blind administration of antimalarial drugs in such subjects will further exaggerate the normal physiology of an individual. Sometimes, it may be fatal also. Therefore, the susceptible and vulnerable communities should be screened for these genetic markers before mass administration of antimalarial (oxidant) drugs in malaria endemic localities or regions in Orissa.[4] It may be concluded that different hemoglobinopathies and G-6-PD deficiency are a fairly common cause and precipitating factors for medicine-induced-hemolytic anemia. Early detection of these genetic disorders and avoiding indiscriminate use of precipitating medicines can prevent drug-induced complications in vulnerable people.[28] The implications of the study are that prior adequate knowledge and awareness of hemoglobinopathies/G-6-PD status of a patient can prevent hemolysis associated morbidity and mortality especially in pregnancy and neonates in a state like Orissa, which has a dubious distinction for the highest infant mortality rate (73 per 1000 live births in the year 2007) in the country.

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