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Protective Resistance by Human G6PD Enzyme Deficiency and Hemoglobin Variants Against Malaria and Natural Selection: Further Evidence from Review of New Studies

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Abstract

Main objective of this article is to review and evaluate recent red cell variant studies for protection against malaria and natural selection. Malaria is a parasitic disease highly widespread in tropical and subtropical regions of the world. It is also one of the leading causes of death worldwide and genes involved in malaria resistance are the most important for natural selection in human populations. Multiple red cell variants, which evolved probably to counter the lethal effects of malaria and confer protection against malaria through different mechanisms, show high frequencies in malaria endemic vulnerable populations. Different natural protective/resistance mechanisms including hampering of parasite growth, invasion related immunological responses or rapidly elimination of malaria parasite from the infected erythrocytes of host have briefly been discussed, evaluated, and reviewed. Conclusions drawn have been projected here. High frequency of inherited hemoglobin disorders including thalassemias, and red cell G6PD enzyme deficiency, which seemed to evolve simultaneously in relation to malaria, and high mortality caused by Plasmodium falciparum malaria in different vulnerable populations of tropical and subtropical parts of world, confirm that the natural selection is certainly operating against malaria in one way or another; and human population genetics have distinctly played a significant role in the co-evolution of host and malaria. The inverse relationship between sickle cell trait and G6PD deficiency and vice versa, revealed by allele frequencies distribution shown in our previous studies, is a testimony of disequilibrium, as sickle cell allele being replaced by G6PD deficiency allele in populations of central India. Positive natural selection plays a definite role against malaria for maintaining balance in high frequency endemic populations.

Keywords: G6PD deficiency, Hemoglobin variants, Thalassemia, Protective resistance, Inverse relationship, Malaria, Natural selection.

Abbreviations: SCA-Sickle Cell Anemia, G6PD-Glucose-6-Phosphate Dehydrogenase, NSAIDs-Nonsteroidal Anti-Inflammatory Medications.

Introduction

There is a need to recognize and respect the fact that each person is a unique in this world with its gifted qualities and genetic endowments. Every human-being fortunately or unfortunately needs to be aware of the fact that the present human body shape, size and figure is going to give up one day or another day and what will stay in the world, are the novel deeds, enlightening thoughts, genuine and truthful contributions and distinguished achievements during the life span. Human health and disease are the relative terms which depend on the healthy and balanced up keep against the odd surrounding conditions. When the human populations are subject to dwelling in varied geography, ecology, different environmental conditions, climatic fluctuations and changes, disease susceptibility, persistence of diseases, or bio-social inherent pressures, the natural selection may alter the genetic allele frequencies in one population relative to another [1,2]. Positive natural selection the phenomenon that accounts for the increase in the prevalence of advantageous traits in a population has played an important role in human development and evolution as a species. Large differences in allele frequencies between populations, thus, are the signals in the genome that have undergone selection.

Other signals of recent positive selection include the long haplotypes, and reduced allelic variation in the regions around the selected variants. Therefore, the characterization of signatures of positive selection in genes, that are of adaptive significance in human populations, have greater medical relevance for identifying the functionally significant variants that play important roles in health and disease scenario of the host [1,2].

Moreover, different persons may differ in genetic constitution and its response to an infectious disease for example, the malaria. Malaria has been one of the most prevalent and successful parasitic diseases widely spread throughout the globe. Plasmodium parasites, due to multiple factors, have complex biology, high polymorphism, increasingly high resistance to anti-malarial drugs in many endemic regions of the world. As a result of interaction between malaria parasites and human species have led to fixation of several inherited alterations in many populations so that some of the underlying mechanisms confer protection against malaria [1,2]. Natural selection supports such positively involved struggle for existence of the fittest of all.

Cardinal Malaria Situation

Malaria is a parasitic disease highly widespread in tropical and subtropical regions of the world. This disease is most commonly found in poor countries, having less developed systems for health infrastructure, and inadequate control and comprehensive preventive strategies. High rates of morbidity and mortality can be attributed to the lack of timely available diagnostic facilities, due to financial constraints, and geographical and transport barriers, access to effective treatment due to insufficient supply of quality medicine, and the growing parasite resistance to anti-malarial drugs such as chloroquine, pyrimethamine, etc. The immune response induced in humans by parasitic infection of malaria is a complex one and it varies depending on genetic make-up of the host, age, epidemiological factors, level of malaria endemicity, parasite stage, parasite species, availability of diagnostic facilities and quality of treatment, and repeated parasitic infection. Both innate and acquired immunity processes are invoked vigorously during the infection.

The World Malaria Report 2017 presents a comprehensive state of play in global progress in the fight against malaria up to the end of 2016. In 2016, an estimated 216 million cases of malaria occurred worldwide. Most malaria cases in 2016 were in the World Health Organization (WHO) African Region (90%), followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%) and the like. Plasmodium falciparum is the most prevalent malaria parasite in sub-Saharan Africa, accounting for 99% of estimated malaria cases in 2016. In 2016, there were an estimated 445,000 deaths from malaria globally. About 2 million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia. India was fourth with 7 percent of deaths, after Nigeria (30%), the Democratic Republic of Congo (14%), Burkina Faso (7%) and so on [3].

Malaria is one of the leading causes of death worldwide and genes involved in malaria resistance are the most important for the natural selection in human populations. In 1949, Haldane suggested that infectious disease could be a strong selective force in human populations [4]. Evidence for the strong selective effect of malaria resistance includes the high frequency of a number of detrimental hemato-genetic diseases (including the different hemoglobinopathies, thalassemias, and red cell enzymopathy), caused by the effects of these malaria resistance variants. In view of this pathetic scenario, it is justifiable that population genetics could be useful to determine the amount and pattern of natural selection in human population isolates [5-12].

Human Red Cell Genetic Variants and Plasmodium Falciparum Malaria

Red cell variants that modulate malaria risk can serve as models to identify clinically relevant mechanisms of pathogenesis, and thus define parasite and host targets for next-generation therapies. Multiple red cell variants are known to confer protection from malaria. From a biological point-of-view, these insights highlight the co-evolution of host and parasite and serve as a model of balancing selection. From a clinical perspective, these relationships represent a naturally occurring model of protection from severe, life threatening malaria, which can be used to isolate the mechanisms of parasite pathogenesis. By preventing malaria parasites from causing disease, these red cell variants could help discover clinically significant mechanism(s) of pathogenesis and investigating them as targets for future therapeutics.

Thalassemia

JBS Haldane was the first who speculated the Darwinian (Natural) selection that, depending on the genetic make-up, the people would have a different risk of dying when they are confronted by a parasitic organism; so much so that, even if, a gene offering protection against those parasites were, otherwise, harmful, its frequency would increase when a population was exposed to the parasites [4,13]. Later, Haldane hypothesized that one important example could be of thalassemia in the face of malaria for several reasons. First, one type of malaria, caused by Plasmodium falciparum, is highly lethal. Second, it is estimated to have been spread in many parts of the world for several thousands of years, i.e. for several hundreds of generations; thus, malaria as an agent of natural selection seemed to be a better candidate than an infectious disease, causing occasional epidemics, even if associated with high mortality (such as influenza or AIDS). Third, deaths from malaria take place mostly in children, i.e. before reproduction, a critical criterion for effective selection. Last but not the least, Plasmodia take on different forms in the course of their life cycle, but what causes a disease, are the intra-erythrocyte parasites. Therefore, in principle, it is not surprising that, if red cells are in any way abnormal (as they are, for instance, in thalassemia), they may affect the chance of success of the parasites [14].

Similarly, alpha (α+)-thalassemia, being very common in malaria endemic regions, it has been considered to confer protection against clinical disease caused by severe forms of Plasmodium falciparum malaria infection. In the same way, beta (β+)-thalassemia provides protection against the Plasmodium falciparum malaria with significantly lower growth of malaria parasite inside the infected erythrocytes and higher phagocytosis of β-thalassemic infected erythrocytes when compared to normal infected erythrocytes. Moreover, the resistance given to malaria parasite inside the infected erythrocytes is almost identical to that of sickle cell trait infected erythrocytes.

Sickle Cell Anemia

This brief review has been focused on the close and complex relationship of blood disease, e.g. the Sickle Cell Anemia (SCA) with infectious disease of malaria. Sickle cell anemia is a major hemolytic anemia and its epidemiology represents a remarkable signature of the past and present world distribution of Plasmodium falciparum malaria [14]. On one hand, heterozygotes (Hb AS) for the sickle gene are relatively protected against the danger of dying with malaria as now firmly established through a number of clinical field studies reviewed from different parts of Africa, South East Asia, Indian Sub-continent, and the Middle East regions [11,15-20]. In addition, the experimental work is consistent with the heterozygote (Hb AS) red cells infected with Plasmodium falciparum are preferentially removed by a mechanism of macrophages [17,19]. On the other hand, patients homozygous for the sickle gene, suffer from sickle cell anemia, are highly susceptible to the lethal effects of malaria [14].

The simplest explanation of this fact is that malaria makes the anemia of homozygous (Hb SS) cells more severe; leading to often hyposplenism, which reduces the clearance of parasites. From public health point of view, it is important that in malaria-endemic countries, the patients with sickle cell anemia, and particularly the children, are being protected from malaria by appropriate prophylaxis [14,21,22]. Since the humans, like most animals, are diploid, therefore, have more options in this respect. Sickle cell anemia is a disease of homozygotes (Hb SS)-that's why it is called recessive disease-whereas, heterozygotes (Hb AS) are normal for most intents and purposes. The first test of Haldane's hypothesis was carried out by Allison when he showed not only that the S gene was frequent in areas of high malaria transmission, but also that AS (Hb) heterozygotes seemed to have less malaria [4,23].

By the laws of population genetics it is to be expected that wherever the sickle (S) gene is common, there will be many patients suffering from sickle cell anemia, a severe burden in the population [21,24]. However, in the same population a much larger number of heterozygotes (Hb AS) will have the advantage of being 'malaria-resistant'. The disadvantage of homozygotes (Hb SS) coexisting with the advantage of heterozygotes (Hb AS)-therefore called a balanced

Tribe	District	N	Sickle cell trait		G6PD Deficiency			
					Male		Male+Female	
			n	%	n	%	n	%
Primitive Tribe								
Bondo	Malkangiri	962	7	0.7	-	-	6	0.6
Paudi Bhuyan	Sundargarh	379	0	0	30	8.1	52	13.8
Didayi	Malkangiri	1014	32	3.2	-	-	16	1.6
Juang	Keonjhar	1065	29	2.7	-	-	46	4.3
Kutia Kondh	Kandhamal	65	2	3.1	4	7.1	4	6.1
Lodha	Mayurbhanj	78	0	0	3	5.3	4	5.1
Saora	Gajapati	177	13	7.3	14	8.9	14	7.9
Lanjia Saora	Gajapati	74	9	13.2	5	7.9	5	6.8
Sabar	Ganjam	102	2	2	9	9.5	9	8.8
Scheduled Tribe								
Bathudi	Mayurbhanj	95	1	1	4	8.2	9	9.5
Bhatra	Nawarangpur	166	25	15.1	3	4.3	11	6.6
Bhumiz	Mayurbhanj	116	1	0.9	9	12.2	11	9.5
Bhuyan	Sundargarh	92	0	0	5	16.7	7	12.9
Bhuyan	Sundargarh	836	20	2.2	84	10	137	16.4
Paraja Bhuyan	Sundargarh	213	2	0.9	29	13.6	45	21.1
Paik Bhuyan	Sundargarh	244	18	7.3	25	10.2	40	16.4
Gond	Kalahandi	219	46	21	12	8.5	13	5.9
Kharia	Sundargarh	54	4	7.4	1	12.5	5	14.2
Kharia	Sundargarh	767	41	5.4	91	11.9	187	24.4
Dudh Kharia	Sundargarh	422	0	0	36	8.5	81	19.2
Dhelki Kharia	Sundargarh	345	41	11.8	55	15.9	106	30.7
Kissan	Sundargarh	130	0	0	3	5.5	5	5.1
Kolha	Mayurbhanj	102	0	0	9	10.7	10	9.8
Kondh	Kandhamal	254	8	3.1	11	6.2	17	6.7
Munda	Sundargarh	96	3	3.1	11	15.9	14	15.9
Oraon	Sundargarh	104	0	0	5	8.6	6	8.2
Paraja	Koraput	176	23	13.1	21	17.4	28	15.9
Santal	Mayurbhanj	100	1	1	6	7.7	9	9

Note: *Data from Reference 2.

Table 1: Distribution of sickle cell trait and G6PD deficiency in 18 major scheduled tribes of Odisha, India.

polymorphism. With the S gene, it became clear that balanced polymorphism, in which the severe disease of homozygotes (Hb SS or SCA) is balanced by the advantage of Hb AS heterozygotes, is a reality also in the human species **(Tables 1-4)**.

Several studies have suggested that high prevalence of sickle cell heterozygotes (Hb AS) in several populations is a protection mechanism against Plasmodium falciparum malaria [1,2,8,10,23]. Moreover, erythrocyte containing Hb S inhibit malaria parasite growth in vivo and confer/provide protection against severe malaria. The following points have emerged from the above studies: (i) Sickle cell (Hb AS) heterozygotes do get malaria. (ii) Hb AS heterozygotes with malaria tend to have lower numbers of parasitized red cells in their blood. (iii) Hb AS heterozygotes have a decreased incidence of the two forms of severe malaria recognized as immediately life-threatening: namely, cerebral malaria and malaria with severe anemia. (iv) Hb AS heterozygotes very rarely die of malaria, even in the rare cases when they do develop cerebral malaria [25].

It is justified that point 4 (iv) is a consequence of point 3 (iii), and point 3 (iii) is at least to some extent the result of point 2 (ii). From clinical epidemiology point of view, the above logistics are consistent with increased fitness of AS heterozygotes in malaria endemic environment (there would be no advantage without malaria) and Hb S is not an absolute impediment to the malaria parasite [17]. Therefore, the mechanism for the increased fitness of Hb AS heterozygotes is not failure of invading red cells, but once the parasite has triggered sickling-enhanced-removal of parasitized sickle cells would take place by macrophages [26]. Thus, the invasion of Plasmodium falciparum on Hb AS heterozygote cells amounts to suicidal infection and the parasitized sickle cells phagocytose rapidly. The clinically relevant consequence of this process is to keep parasitemia relatively lower in Hb AS heterozygotes, and probably the same mechanism applies also to parasitized G6PD deficient red cells [27-32].

These mechanisms include accelerated sickling of parasite infected (Hb AS) erythrocytes, low growth rates, and parasite invasion in (Hb AS) erythrocytes (in low oxygen conditions), and increased phagocytosis of infected (Hb AS) erythrocytes. However, the experimental data support only: 1) Intra-erythrocyte parasite growth is greatly inhibited by sickle cell polymerization when oxygen levels drop below 5% and 2) Higher parasite infected sickle erythrocytes phagocytosis by host immune cells has been observed when compared to infected normal erythrocytes [27,33]. It has also been shown that Hb S erythrocytes infected by Plasmodium falciparum lower the surface expression of PfEMP-1, which results in a reduction of cyto-adherence and thus, providing protection against severe malaria [34].

Clinically, malaria makes the anemia of homozygous sickle cell disease cases worse to the point of becoming life-threatening; and like any other acute infection, malaria can trigger sickle cell anemia in patients, leading to a pain crisis or a sequestration crisis **(Tables 2-3)**. Normally the spleen plays an important role in filtering and removing parasitized red cells. But patients with SCA regularly have an impaired splenic function; often to the extent of functional asplenia, and sometimes the functional asplenia evolves to anatomical atrophy of the spleen from multiple infarcts, so-called auto-splenectomy and the

patients ought to be protected by life-long anti-malarial prophylaxis [35,36]. Distinct mechanisms conferring protection against severe and complicated malaria have been proposed for different hemoglobinopathies such as sickle cell trait (Hb AS), sickle cell anemia (Hb SS), α- and β-thalassemias by several investigators

Tribe	District	N	Sickle cell trait		G6PD Deficiency			
			Male		Male+Female			
			n	%	n	%	n	%
Primitive Tribe								
Birhor	Raigarh	270	0	0	-	-	0	0
Hill Korwa	Jashpur	744	13	1.7	32	6.4	-	-
Hill Korwa	Surguja	402	0	0	-	-	6	1.6
Kamar	Raipur	320	3	0.9	-	-	5	1.6
Kawar	Ambikapur	114	1	0.9	22	16	-	-
Kawar	Raipur	72	4	5.5	25	21.5	-	-
Hill Maria	Bastar	93	21	22.5	-	-	3	3.6
Maria	Bastar	94	19	20.2	-	-	0	0
Maria	Bastar	101	14	13.9	-	-	14	14
Muria	Bastar	101	15	14.9	-	-	3	2.7
Scheduled Tribe								
Bhatra	Bastar	102	10	9.8	-	-	3	2.9
Bhatra	Bastar	99	13	13.1	-	-	0	0
Dhurwa	Bastar	81	5	6.2	-	-	0	0
Gond	Ambikapur	127	26	20.5	22	13	-	-
Gond	Raipur	157	25	15.9	46	16.2	-	-
Halba	Raipur	122	17	13.9	8	7.4	-	-
Halba	Bastar	99	12	12.1	-	-	4	3.6
Halba	Rajnandgaon Or Durg	365	54	15.6	-	-	4	1
Halba	Bastar	95	18	19	-	-	2	1.8
Kodaku	Surguja	400	12	3	-	-	7	1.8
Oraon	Ambikapur	422	9	2.1	55	13.4	-	-
Oraon	Raigarh, Surguja	215	0	0	-	-	0	0
Pando*	Surguja	458	5	1.1	-	-	5	1.1

Note: *A case of hemoglobin AE was detected. *Data from Reference 2.

Table 2: Distribution of sickle cell trait and G6PD deficiency in scheduled tribe communities of Chhattisgarh, India.

[2,16,17]. High fetal and childhood mortality have also recently been described [37,38]. Among the most relevant mechanisms, reduced erythrocyte invasion by the parasite, decreased intra-erythrocyte parasite growth enhanced phagocytosis of parasite-infected erythrocytes and increased immune response against parasite infected erythrocytes have been described [27,31,39,40]. Thus, malaria and sickle cell anemia are still major challenges, being the major public health problems. Patients with sickle cell anemia carry the genetic burden that has helped human populations to survive in malaria-endemic regions of the world. The protective effect of the Hb S gene against malaria is one of the best documented examples in the human species of balanced polymorphism, in which the severe disease of homozygotes (Hb SS or SCA) is balanced by the advantage of Hb AS heterozygotes (Tables 1-4).

Hemoglobin C Disease

The hemoglobin variants/mutants, namely, Hb C and Hb S, are known to protect carriers from severe falciparum malaria. There is a malaria protection-inducing mechanism, that intra-erythrocyte parasite growth becomes reduced in individuals having Hb C erythrocytes in both mild and severe malarial infection. Individuals homozygous for Hb CC display a reduced risk of having severe or non-severe infection by Plasmodium falciparum malaria [17,41].

Hemoglobin E Disease

Heterozygous form of hemoglobin E confers protection against severe malarial episodes because there is reduced erythrocyte invasion by merozoites, lower intra-erythrocyte parasite growth, and enhanced phagocytosis of infected erythrocytes [42-44].

Cytoskeletal Abnormalities

Hereditary spherocytosis (also known as Minkowski–Chauffard syndrome) is an abnormality of erythrocytes. The disorder is caused by mutations in genes relating to membrane proteins that allow for the erythrocytes to change shape. Both Hereditary Ovalocytosis/Ellyptocytosis (Band 3 Variant) and Hereditary Spherocytosis variants reduce the Plasmodium falciparum growth in vitro [45,46]. Ovalocytosis is an uncommon variant of hereditary ellyptocytosis belonging to the erythrocyte membrane inherited disorder. Only the heterozygotic form, which is asymptomatic and endemic in Southeast Asia, derives its name as Southeast Asian ovalocytosis [41]. It gives protection against cerebral malaria.

Glucose-6-Phosphate Dehydrogenase Enzyme Deficiency

The enzyme G6PD deficiency is a genetic disorder that occurs almost exclusively in males. This condition mainly affects red blood cells, which carry oxygen from lungs to various tissues throughout the body, resulting in reduced oxygen flow to the different organs. This can cause fatigue, yellowing of the skin and eyes, and shortness of breath. Additional symptoms of G6PD deficiency include:

- rapid heart rate
- shortness of breath
- urine is dark or yellow-orange
- fever
- fatigue
- dizziness
- paleness
- jaundice, or yellowing of the skin and whites of the eyes

The G6PD enzyme deficiency results from mutations in the G6PD gene. The mutation reduces the amount of G6PD enzyme or alters its structure, so that enzyme can no longer play its protective role. As a result, reactive oxygen species can accumulate and damage red blood cells. Factors such as infections, certain drugs, or ingesting fava beans can increase the levels of reactive oxygen species, causing destruction of erythrocytes faster than the body can replace them. A reduction in number of red blood cells causes the signs and symptoms of hemolytic anemia. G6PD enzyme involves in the normal processing of carbohydrates metabolism. It also protects red blood cells from the

Tribe	District	N	Sickle cell trait		G6PD Deficiency			
					Male		Male+Female	
			n	%	n	%	n	%
Primitive Tribe								
Baiga	Mandla	1566	244	15.6	25	4.5	-	-
Baiga	Dindori	990	182	18.4	-	-	34	3.4
Baiga	Shahdol	219	23	10.5	-	-	4	1.8
Bharia	Chhindwara (Inside)	183	24	13.2	-	-	22	12.1
Bharia	Chhindwara (Outside)	102	3	2.9	-	-	9	8.3
Scheduled Tribe								
Barela	Khargone	345	88	25.5	18	5.8	-	-
Barela	Nimar	316	86	27.2	-	-	18	5.7
Bhil	Jhabua	904	183	20	8	6.7	-	-
Bhil	Ratlam	433	51	11.8	15	3.4	-	-
Bhil	Nimar	316	45	14.2	-	-	34	4.5
Bhilala	Jhabua	403	123	30.5	23	7.2	-	-
Bhilala	Nimar	370	68	18.4	-	-	16	4.3
Gond	Betul	299	34	11.4	-	-	9	3
Gond	Damoh	321	106	33	-	-	30	9.3
Gond	Jabalpur	3224	612	19	-	-	0	0
Gond	Mandla	280	52	18.6	-	-	0	0
Gond	Chhindwara (Inside)	75	15	4.3	-	-	3	3.7
Gond	Chhindwara (Outside)	158	25	15.8	-	-	5	3.1
Gond	Chhindwara	83	10	12	-	-	3	3.1
Gond	Seoni	286	54	18.9	-	-	5	1.7
Gond	Shahdol	252	33	13.1	-	-	8	3.2
Gond	Balaghat	311	48	15.4	-	-	4	1.3
Kol	Satna	290	12	4.1	-	-	13	4.5
Korku	Chhindwara	250	43	17.2	-	-	14	5.6
Korku	Khandwa	301	51	16.9	-	-	4	1.3
Korku	Betul	296	41	13.8	-	-	8	2.7
Panika	Shahdol	210	60	28.6	-	-	6	2.8
Pradhan	Dindori	990	182	18.4	-	-	11	4.9
Patelia	Jhabua	166	34	20.5	-	-	4	2.6
Raj Gond	Damoh	321	33	10.3	-	-	96	30
Balai	Khandwa	276	39	14.1	-	-	2	0.7
Basod	Betul	123	24	19.5	-	-	3	2.4
Basod	Chhindwara	150	33	22	-	-	0	0
Choudhury (Chamar)	Damoh	339	61	18	-	-	6	1.8
Choudhury (Chamar)	Shahdol	195	10	5.1	-	-	5	2.6
Jharia	Jabalpur	637	28	4.4	-	-	0	0
Jharia	Jabalpur	409	155	37.9	-	-	0	0
Katiya	Chhindwara	181	45	24.9	-	-	7	2
Mehra	Betul	352	114	32.4	-	-	7	2
Mehra	Chhindwara	114	23	19.8	-	-	3	2.6
Mehra	Seoni	216	46	21.3	-	-	4	1.8
Mehra	Balaghat	219	40	18.3	-	-	3	1.4
Other Backward Class	Chhindwara	58	7	12.1	-	-	4	6.9

Note: *Data from Reference 2.

Table 3: Distribution of sickle cell trait and G6PD deficiency in scheduled caste and scheduled tribe communities of Madhya Pradesh, India.

effects of potentially harmful molecules called reactive oxygen species by products of normal cellular functions. Chemical reactions, involving G6PD, produce compounds that prevent reactive oxygen species from building up to toxic levels within red blood cells. In affected individuals, a defect in G6PD enzyme causes red blood cells to break down, called hemolysis, prematurely faster than the body can replace them [30]. Once G6PD deficiency has progressed to hemolytic anemia, more aggressive treatment may be required. This usually includes oxygen therapy and blood transfusion to replenish oxygen and red blood cells. The affected person will need to stay in the hospital, while receiving these treatments as close monitoring required of severe hemolytic anemia, and is critical for ensuring a full recovery without complications. In people with G6PD deficiency, hemolytic anemia is most often triggered by bacterial or viral infections or by certain drugs (such as some antibiotics and medications used to treat malaria).

Hemolytic anemia can also occur after eating fava beans or inhaling pollen from fava plants (a reaction called favism) [2,9]. It may also be triggered by infections or by certain drugs such as:

- Antimalarials, a type of medication used to prevent and treat malaria

Tribe	District	Sickle Cell Trait*		N	G6PD Deficiency				
					Male		Male+Female		
		n	%		n	%	n	%	Reference
Scheduled Tribe									
Andha	Nanded	-	2						
Bhil	Nandurbar	-	20.6	215	-	-	16	7.4	2
Gond, Madia	Gadehiroli Yeotmal	-	20.8	135	-	-	24	19.4	2
Halbi	Gadchiroli	-	13.9						
Katkari	Pune, Raigad, Ratnagiri	-	5.9	77	-	-	6	7.8	2
Kokana	Dhule, Nasik	-	3.5	83	-	-	7	8.4	2
Kolam	Yeotmal	-	8.3						
Korku	Amravati	-	9.5						
Mahadeo Koli	Pune, Nasik	-	0.8						
Malhar Koli	Thane	-	13.9	687	-	-	21	3.1	2
Otkar	Gadchiroli	-	35						
Pardhan	Nanded, Yeotmal	-	33.7						
Pawara	Dhule, Jalgaon	-	25.2	87	-	-	3	3.5	2
Raj Gond	Gadchiroli	-	10.9						
Tandvi	Jalgaon	-	8.3						
Thakur	Ahmednagar, Pune, aigad, Thane	-	0						
Warli	Thane	-	8	656	-	-	112	17	2
Warli	Thane			128	-	-	25	19.5	2

Table 4: Distribution of sickle cell trait and G6PD deficiency in scheduled caste and scheduled tribe communities of Maharashtra, India.

- Sulfonamides, a medication used for treating various infections
- Aspirin, a drug used for relieving fever, pain and swelling
- Some Non-steroidal Anti-Inflammatory Medications (NSAIDs).

Once the underlying cause is treated or resolved, symptoms of G6PD deficiency usually disappear within a few weeks. G6PD deficiency is also a significant cause of mild to severe jaundice in newborns. Many people with this disorder, however, never experience any signs or symptoms and are unaware that they have the condition.

On the other hand, age specific mortality is high, i.e., as the age advances, the number of G6PD deficiency individuals go on decreasing in a malaria endemic populations, has been reported by some investigators [32]. The G6PD enzyme deficiency is inherited as an X-linked recessive pattern. The gene associated with this enzyme deficiency is located on the X chromosome, which is one of the two sex-chromosomes. In males (who have only one X chromosome, in hemizygous condition), one altered copy of the gene in each cell is sufficient to cause the deficiency.

In females (who have two X chromosomes), a mutation would have to occur in both copies (alleles) of the gene to cause the disease. But, daughters always get their X chromosomes from parents, one each from father and mother. Therefore, they may be heterozygote, if father is affected and mother is normal. Similarly, they may also be heterozygote, if one of the X chromosomes is carrier or trait for G6PD deficiency. Further it all depends on the X chromosome, either inherited from affected father or carrier mother, and also on the activation of one (normal or abnormal) out of the mother's two X chromosomes according to the Lyon's Hypothesis [9,10]. Thus, G6PD deficiency would have to occur in both X chromosomes (counter parts) of females (from father as well as from mother) to fully express the defective gene (in homozygous state).

Males are affected by X-linked recessive disorders much more frequently than the females (Tables 1-4). A characteristic of X-linked inheritance is that fathers cannot pass X-linked traits to their sons [47]. In contrast to the findings by Ruwende et al. a later study showed that a form of G6PD deficiency confers protection against severe malaria in its uniform state (hemizygous males and homozygous females) but not in its mosaic state, i.e. heterozygous females [48]. This finding is consistent with those protection mechanisms involving either enhanced phagocytosis or the effects on pathogenic consequences in the microcirculation of parasitized erythrocytes, since both are expected to operate preferentially on uniformly deficient erythrocytes [26].

Red Cell Genetic Variants and Natural Selection against Malaria

High frequency distribution of inherited hemoglobin disorders including thalassemias, and red cell G6PD enzyme deficiency, which have probably evolved simultaneously in relation to malaria in different vulnerable and malaria endemic populations, and high mortality caused by Plasmodium falciparum malaria in different tropical and subtropical parts of the world confirm that the natural selection is certainly operating against malaria in one way or another; and human population genetics play a major role in this process of co-evolution of human-beings and malaria. The people who have a G6PD deficiency mutation may be partially protected against Plasmodium falciparum malaria. The deficiency of G6PD enzyme or a reduction in the amount of functional G6PD appears to make it more difficult for the malaria parasite to invade red blood cells, inhibits its growth, and phagocytises rapidly [49]. G6PD deficiency occurs most frequently in areas of the world where malaria is common. Moreover, disequilibrium of genetic markers such as various variants of hemoglobin and high occurrence of G6PD deficiency is reflected as the natural selection mechanism for protection against malaria [1,2,11,27,31].

Natural selection can maintain deleterious alleles in the population if there is a heterozygote advantage (positive selection) as in the case of sickle cell trait (Hb AS). When the frequency of sickle cell allele decreases in malaria endemic cross-section of the tribal population in India, the frequency of G6PD enzyme deficiency allele increases and vice versa [1,2]. This trend for an inverse relationship between sickle cell disorders and G6PD deficiency due to disequilibrium in major scheduled caste and tribal communities of Central-Eastern India, is fascinating one [1,2]. This medical aspect is important from an evolutionary biological background and could be an excellent starting point for molecular analyses to determine the signature of natural selection in the genomic regions of the β-globin and G6PD genes. This

may further provide a mechanism for how natural selection operates against malaria when two mutations occur in the same geographical region [2].

Similarly, further work on the remarkable epistatic interactions between various malaria-protective polymorphisms could provide invaluable information about the mechanisms for the distribution of the different forms of inherited hemoglobin disorders particularly in high-frequency populations (Tables 1-4). Further natural selection had played a major role initially in favor of sickle cell, β -thalassemia and G6PD mutations so that they have probably evolved as a protective mechanism against the lethal effects of malaria. Since the selection favors the mutation with least cost to the population, as the clinical manifestations of G6PD deficiency are mild and do not result in a complete loss of enzyme activity against the sickle cell disease with high morbidity and mortality in the region, and predominant frequency of G6PD deficiency over the sickle cell disorders in some aboriginal communities in India. It seems that the replacement of the sickle cell allele for G6PD deficiency allele is occurring due to disequilibrium in the major scheduled castes/tribes of Chhattisgarh, Madhya Pradesh, Maharashtra, and Odisha state in Central India [2]. This means that the decrease in sickle cell allele is compensated by the increase of G6PD deficiency alleles. It seems that different abnormal hemoglobin variants (C,D,E,F,S and thalassemias) and G6PD enzyme deficiency are the directed mutations against the malaria malady as the heterozygotes or carriers of these genetic traits do not suffer severely from the dreadful malaria (Tables 1-4).

Another important factor is the relatively high frequency of consanguineous marriages in many regions of India with high frequency of these red cell genetic variants; this mechanism has an important effect on increasing the gene frequency of the fore said recessively inherited disorders in vulnerable populations of central India [50,51]. Although accurate data on the frequency of consanguineous marriages are lacking, there is no doubt that this is an important factor in helping to maintain the global or regional or local health problem posed by the high frequency of red cell genetic variants and malaria conditions, and significantly contributing towards the high morbidity, maternal mortality, and fetal and childhood mortality [21,22,37,38].

The varying distribution of some of the hemoglobin disorders, and G6PD enzyme deficiency reflects strong founder effects of their original inhabitants in different populations [6,8]. Another important factor is the epidemiological conditions, whereby as the public health and nutritional standards improve in the poorer countries, babies with these red cell hemolytic conditions who would, otherwise, have died in early life, are now living long enough to present for diagnosis and management [21]. An estimated 400 million people worldwide have G6PD deficiency. This condition occurs most frequently in certain parts of Africa, Southeast Asia including India, the Mediterranean, and the Middle East. It affects about 1/10 African American males in United States [17,41].

Concluding Comments

The frequency distribution of inherited hemoglobin disorders and Plasmodium falciparum malaria are posing increased burden on human health resources. Their high frequency is a reflection of natural selection combined with a high frequency of consanguineous marriages in many communities and regions, together with an epidemiological expansion due to public health improvement in the affected communities as more babies with these disorders survive to present for treatment in future too. The strongest evidence for Hb S and (α+)- and (β+)-thalassemias, without any doubt, that malaria is responsible for the current distributions of all the major hemoglobin disorders in the world. Malaria is one of the leading causes of death worldwide and has been suggested as the most potent type of selection in humans in recent millennia. As a result, genes involved in malaria resistance are excellent examples of strong selection in recent years. Perhaps best known is the sickle cell hemoglobin variant, which is often used as an example of heterozygote advantage.

In addition, G6PD deficiency illustrates strong selection at an X-linked locus, followed by β-globin variants C, D, E and S variants. In 1949, Haldane initially suggested that infectious disease; specifically the malaria could be a strong selective force in human populations. Evidence for the strong selective effect of malaria resistance includes the high frequency of a number of detrimental genetic diseases caused by the pleiotropic effects of these malaria resistance variants. In contrast, there are many changes that modify levels of expression and provide malaria resistance for G6PD deficiency, α-thalassemia, and β-thalassemia. Malaria parasites have co-evolved with the host and constitute an important deriving evolutionary force behind common erythrocyte variants such as thalassemia, sickle cell disease, Hb C, Hb D, Hb E and G6PD deficiency and other erythrocyte anomalies.
Host-parasite interactions have led to a host's relative resistance to the parasite. There could be two reasons for malaria mediated evolutionary selection:

- Strong selective pressure in case of higher frequency of Hb S allele found in malaria exposed populations; and
- Independent evolutionary responses developed by different populations both at global (e.g. Hb C, Hb D, Hb E and Hb S confer protection against malaria because mutations affect the hemoglobin functionality) and local level (four different HbS haplotypes found in Africa) and the Arab-Indian haplotype is different from the African haplotypes.

Different mechanisms conferring protection against malaria are widely found in different populations of the world, and that the populations have evolved and developed different genetic variants, which are related to resistance to the malaria disease. This could imply that the maintenance of these alleles in the population has been due to the effects of positive selection against the lethal malaria. There also seems to be disequilibrium and competition between two red cell variants, i. e. Sickle cell disease (Hb SS) and G6PD enzyme deficiency. When the frequency of sickle cell allele decreases in malaria endemic tribal population in India, the frequency of G6PD enzyme deficiency allele increases and vice versa.

This trend for an inverse relationship between sickle cell disorders and G6PD deficiency in major scheduled caste and tribal communities of Central-Eastern India, is fascinating one. Since the selection favors the mutation with least cost to the population, as the clinical manifestations of G6PD deficiency are mild and do not result in a complete loss of enzyme activity against the sickle cell disease with high morbidity and mortality in the region. Even though the above could explain, why mutations conferring malaria protection are highly variable and maintained in the population, the association between sickle cell disease and G6PD enzyme deficiency seems to be well-suited here. Thus, the protection is principally present for severe disease and largely absent for Plasmodium falciparum infection, suggesting that hemoglobin disorders specifically neutralize the parasite's in vivo mechanisms of pathogenesis.

These genetic traits-including Hemoglobin C (Hb C), Hemoglobin D (Hb D), Hemoglobin E (Hb E), Hemoglobin S (Hb S) and α- and β-thalassemias-are the most common monogenic human disorders and can confer remarkable degrees of protection from severe, life-threatening falciparum malaria in African children: the risk is reduced 70% by homozygous Hb C and 90% by heterozygous Hb S (sickle-cell trait). These hemoglobin variants thus represent a "natural experiment" to identify the cellular and molecular mechanisms by which Plasmodium falciparum produces clinical morbidity, which remain partially obscured due to the complexity of interactions between this parasite and its human host.

Multiple lines of evidence support a restriction of parasite growth by various hemoglobinopathies, and recent data suggest this phenomenon may result from host micro RNA interference with parasite metabolism. Therefore, owing to the co-evolution of humans and Plasmodium falciparum parasites, the human genome is imprinted with polymorphisms that not only confer innate resistance to falciparum malaria, but also cause hemoglobinopathies to counter the adverse effects of severe malaria.

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