Chapter – 1

INTRODUCTION

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1.1. Glucose-6-Phosphate Dehydrogenase enzyme

Glucose-6-Phosphate Dehydrogenase (G6PD) is an X-linked cytoplasmic housekeeping enzyme (EC 1.1.1.49) serving as a major component of Pentose Phosphate Pathway (PPP). PPP is a metabolic pathway parallel to glycolysis responsible for the production of ribose-5-phosphate sugar and nicotinamide adenine dinucleotide phosphate (NADPH). Ribose-5-phosphate is used for nucleotide synthesis and NADPH is required for protecting the cells from the toxic reactive oxygen species (ROS) which helps restore glutathione, an essential antioxidant of our body (Stincone *et al*., 2015). This pathway occurs in the cytoplasm in two phases- oxidative and non-oxidative (Figure 1). The enzyme G6PD is a catalyst in the oxidation of glucose-6-phosphate into 6-phosphogluconate, a rate limiting step of PPP (Stanton, 2012). The generation of NADPH takes place in the first and third step of this pathway, the first of which is regulated by G6PD. In this step, conversion of $NADP^+$ to its reduced form NADPH is brought about by G6PD. A major function of the RBCs is to provide protection against oxidative stress, mediated through the glutathione cycle. In this cycle, a balanced regeneration of reduced form of glutathione (GSH) is required, which is dependent on a steady supply of NADPH (Figure 2). The process of loading, carrying and unloading of haemoglobin-bound oxygen is done by the Red Blood Cells (RBCs), in which free radicals are formed during the process. Thus, it is very important for the RBCs to protect itself against the endogenous oxidative stress along with other exogenous oxidative stress. Besides G6PD, there are a number of other enzymes that catalyze dehydrogenase reactions in most cells of our body. Therefore, shortage of NADPH does not arise in those cells even when G6PD is scarce. However, in anucleated cells like RBCs, a different situation occurs, due to the fact that the only source for generation of NADPH in the RBCs is G6PD via the PPP.

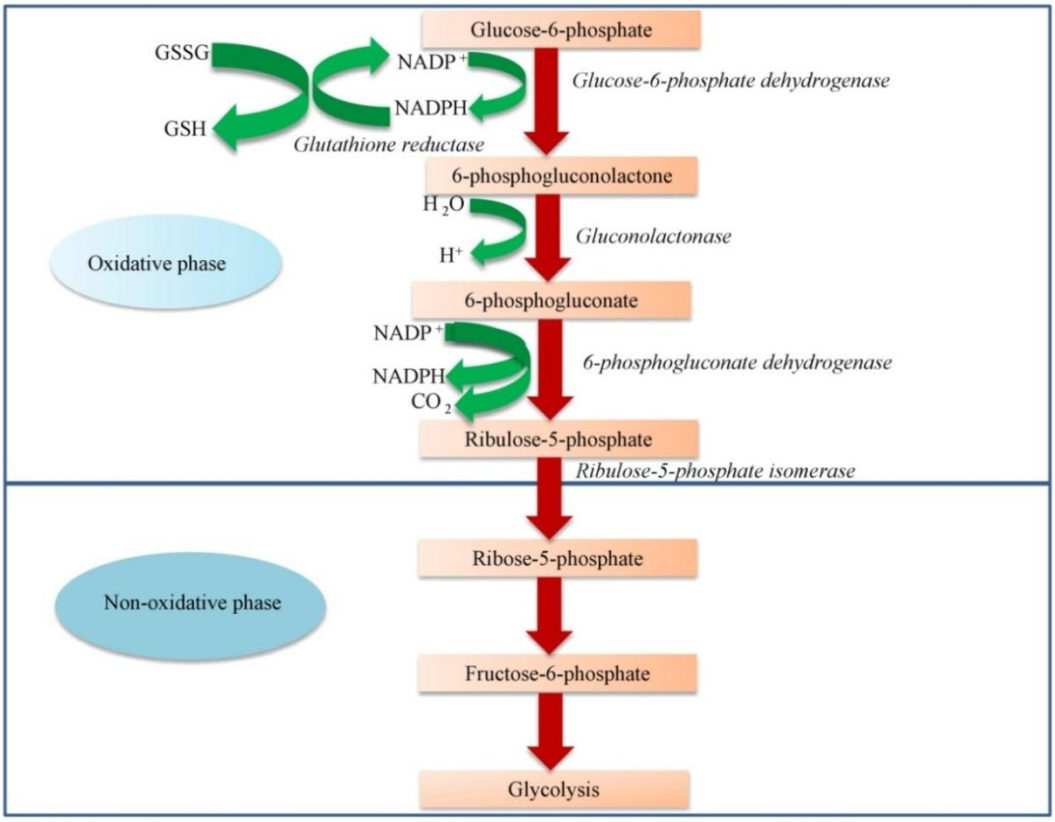

Figure 1. Diagrammatic representation of NADPH generation via Pentose phosphate pathway.

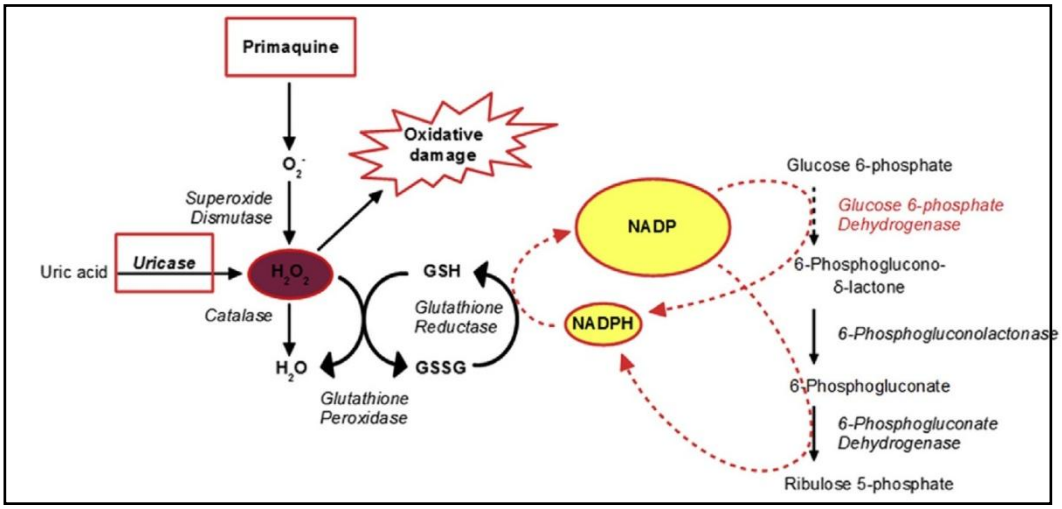

Figure 2. Role of G6PD in defense mechanism against oxidative attack (Luzzatto *et al.*, 2016).

The enzyme G6PD is encoded by the gene *g6pd* located on the distal long arm of the X chromosome (locus q28) as depicted in Figure 3. A total of 13 exons and 12 introns constitute an 18.5 kb long *g6pd* gene (Persico *et al*., 1986). It appears strangely that the first 600bp of the *g6pd* gene's 5' end are not translated. This stretch of base pairs constitutes exon 1 and part of exon 2. The start codon, ATG, is thus found in base 115 of the 127 bp long exon 2 (Gomez-Manzo *et al*., 2016). Depending on the pH, a functionally active G6PD can exist as a dimer or a tetramer. The dimeric form is seen predominantly at physiological pH (Arese *et al*., 2012). Each G6PD monomer consists of two domains: a catalytic nicotinamide adenine dinucleotide phosphate ($NADP^+$)-binding domain and β+α domain, containing an additional binding site for $NADP^+$. The additional $NADP^+$ is essential for the enzyme's structural stability (Kotaka *et al*., 2005; Au *et al*., 2000) (Figure 4). Between these two domains is where the Glucose 6-phosphate (G6P)-binding site is situated. According to a recent study, the majority of variants that lead to severe (10% of normal G6PD activity, class I and class II) or mild (10-60% of normal G6PD activity, class III) deficiency are mostly found in those functional regions of the enzyme, which affects the enzyme's activity and stability (Cunningham *et al*., 2017).

1.2. Glucose-6-Phosphate Dehydrogenase deficiency

More than 400 million individuals globally, or roughly 4.9% of the world's population, suffer from Glucose-6-phosphate dehydrogenase (G6PD) deficiency, a common genetically inherited enzymopathy prevalent in malaria-endemic areas (Nkhoma *et al*., 2009). In malaria endemic regions of the world, G6PD deficiency is very common. Most of the time, people with G6PD deficiency appears normal until they are exposed to haemolytic triggers. These triggers include fava beans, some medications like analgesics and antimalarials, as well as chemicals like napthalene (Peters & Van Noorden, 2009). G6PD deficient people are mostly asymptomatic; however, they can exhibit some clinical symptoms including persistent non-spherocytic haemolytic anaemia, severe neonatal jaundice, and acute haemolytic episodes. Avoiding exposure to the oxidative triggers is the best method for persons with G6PD deficiencies to live normally. A wide range of mutations, primarily single nucleotide substitutions, deletions, and multiple mutations are

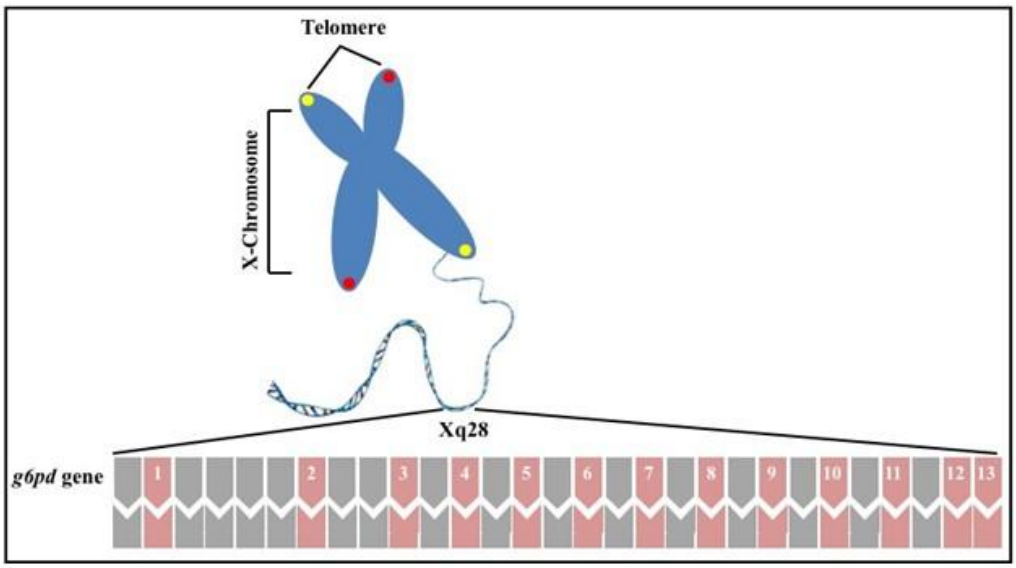

Figure 3. Location of *g6pd* gene (Gomez-Manzo *et al*., 2016).

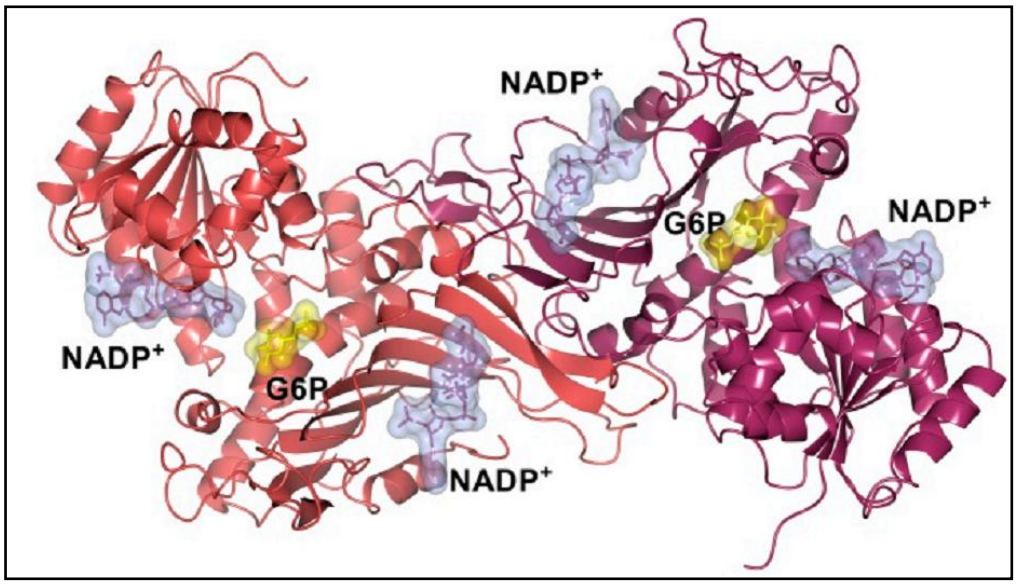

Figure 4. Crystal structure of G6PD enzyme in dimeric form (Gomez-Manzo *et al*., 2016).

the causes of this deficiency (Minucci *et al*., 2012).

1.3. Glucose-6-Phosphate Dehydrogenase variants

Over 450 genotypic variations of G6PD have been reported on the basis of enzyme kinetics, physicochemical features, mutation location and base pairs involved. However, only a small number of variants, about 220 have been molecularly described. The major polymorphic G6PD variants are Mediterranean, Gaohe, Honiara, Aures, Mahidol, Coimbra, Seattle, Montalbano, Viangchan, Cassano, Union, Canton, and Kaiping (Mason *et al*., 2007). The Mediterranean variant (188 Ser→Phe), which is widespread in the Mediterranean regions (Spain, Italy, Greece), the Middle East, and the Indian subcontinent, is the most routinely encountered variant worldwide (Vives-Corrons *et al*., 1990; Kurdi-Haidar *et al*., 1990). Almost all known mutations have been discovered to affect the gene's coding regions, while very few have been found in the non-coding regions (Minucci *et al*., 2012). From India, altogether 27 distinct G6PD variants have been reported till now (Figure 5). The commonly reported variants from India are G6PD Mediterranean, Orissa, Kerala/Kalyan, A^-, Chatham, Mahidol in different populations (Mason *et al*., 2007).

Very few studies have been conducted on G6PD variants from the North-eastern region of India. G6PD Mahidol, Acores, A^+, $A^{-(202)}$ are the variants reported from different population groups of Northeast India (Bharti *et al*., 2019; Rajkhowa *et al*., 2020). Recently, G6PD Mediterranean, Orissa and Kalyan-Kerala have been reported from the ethnic population of the region (Basumatary *et al*., 2023).

World Health Organization (1989) has categorized G6PD variants into 5 classes based on the enzyme activity and related clinical symptoms as shown in Table 1. All of the variants show varying degrees of deficiency, and hence the variants' susceptibility to oxidative haemolysis is also affected by their genotypes (Awab *et al*., 2021).

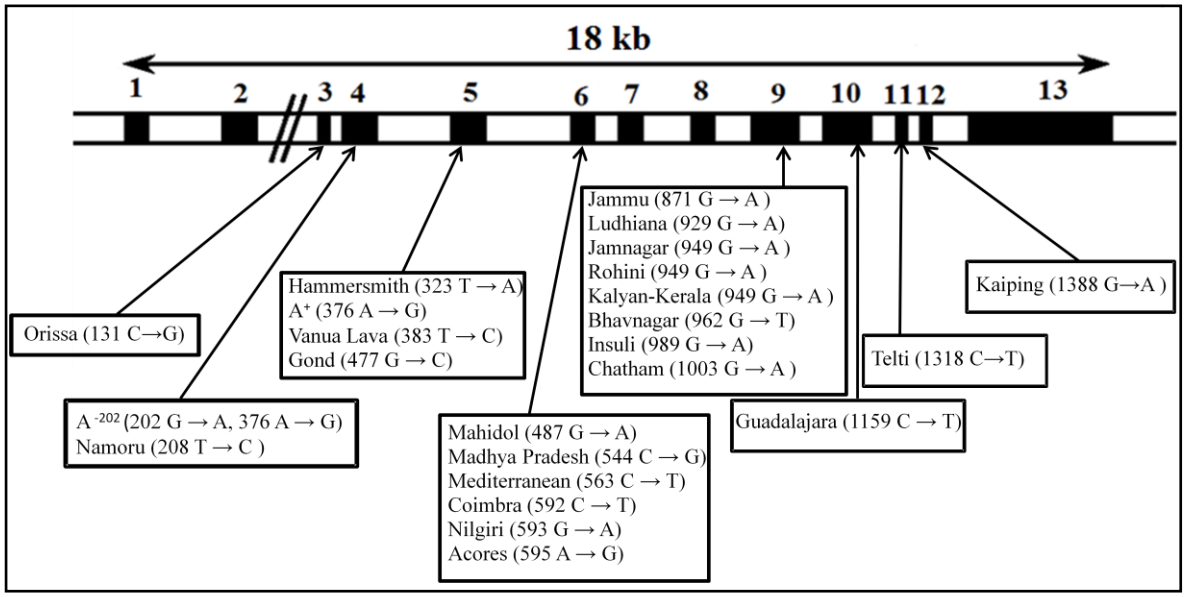

Figure 5. Graphical representation of *g6pd* gene with reported mutations from India.

Table 1. Classification of G6PD variants by WHO (1989).

Class	Enzyme activity	Clinical expression
I	< 10%	Chronic non-spherocytic haemolytic anaemia
II	< 10%	Acute haemolytic anaemia
III	10-60%	Acute haemolytic anaemia
IV	60-150%	No haemolysis
V	> 150%	Uncertain clinical significance

1.4. Haemolysis in Glucose-6-Phosphate Dehydrogenase deficiency

1.4.1. Oxidative triggers in Glucose-6-Phosphate Dehydrogenase deficiency

Haemolysis may occur in people with G6PD deficiency due to various medications and food triggers. Antimalarial medications, sulfonamides, and methylene blue are important initiators of G6PD-related haemolytic crisis (Beutler, 1996; Luzzatto & Seneca, 2014). Individuals with different G6PD variants have different reactions to drug triggered

haemolytic response (Chan *et al*., 1976). Most drug-induced haemolytic anaemia in G6PD-deficient people is caused by primaquine, an anti-malarial medication. The list of the drugs that trigger haemolysis in G6PD deficiency are presented in Table 2. The *Lawsonia inermis* (henna) has also been documented to produce haemolytic crisis by topical application, in newborns with G6PD deficiency (Raupp *et al*., 2001).

Table 2. List of drugs to be avoided by G6PD deficient patients (Frank, 2005).

Sl. No.	**Drug name**	**Use**
1	Primaquine	Antimalarial
2	Dapsone	Anti-infective used for treatment of leprosy
3	Flutamide	Non-steroidal antiandrogen used for prostate cancer treatment
4	Mafenide cream	Topical antimicrobial for treatment of severe burn injuries
5	Methylene blue	Antidote for drug-induced methaemoglobinemia
6	Nalidixic acid	Antibiotic used primarily for treatment of urinary tract infections
7	Nitrofurantoin	Antibiotic used for treatment urinary tract infections
8	Phenazopyridine	Pain-killer used in treatment of dysuria
9	Rasburicase	Adjunct to antineoplastic agents
10	Sulfacetamide	Antibiotic used in ophthalmic and topical preparations
11	Sulfamethoxazole	Antibiotic used in combination preparations
12	Sulfanilamide	Antifungal agent used in treatment of vulvo-vaginal *Candida albicans* infection

There are certain drugs that can be used with caution in G6PD deficient patients. Table 3 summarizes the drugs and their uses that can be used in G6PD deficient patients who do not have non-spherocytic haemolytic anaemia.

Table 3. List of drugs to be used with caution in G6PD deficient patients (Beutler, 2007; Younster *et al*., 2010; Bubp *et al*., 2015).

Sl. No.	Drug name	Use
1	Acetaminophen (Tylenol)	Used for treatment of mild to moderate pain and in reducing fever.
2	Acetylsalicylic acid (aspirin)	Used to treat pain and fever.
3	Antazoline (Antistine)	Antihistamine agent used in treatment of nasal blocking and allergic conjunctivitis.
4	Antipyrine	Used for treatment of pain and swelling of ear.
5	Ascorbic acid (vitamin C)	Used for improving low Vitamin C levels.
6	Benzhexol (Artane)	Used in treatment of muscle spasms, stiffness, tremors and muscle control.
7	Chloramphenicol	Antibiotic used for treatment of eye infections.
8	Chlorguanidine (Proguanil, Paludrine)	Used for prevention and treatment of malaria.
9	Chloroquine	Used for prevention and treatment of malaria.
10	Colchicine	Used in treatment of inflammation and pain.
11	Diphenyldramine (Benadryl)	Used for treatment of hay fever, allergy and common cold.
12	Glyburide (glibenclamide, Diabeta, Glynase)	Antidiabetic agent used for treatment of type II diabetes.
13	Isoniazid	Antibiotic used in treatment of tuberculosis.
14	L-Dopa	Used in treatment of Parkinson disease.
15	Quinine	Antimalarial agent used in treatment of malaria.
16	Streptomycin	Used as an antibiotic medication.

17	Sulfacytine	Used for treatment of acute urinary tract infections.
18	Sulfaguanidine	Used in veterinary medication.
19	Sulfamethoxazole (Gantanol)	Used in combination with trimethoprim for treatment of urinary tract infection, bronchitis, middle ear infection, traveler's diarrhea.
20	Sulfisoxazole (Gantrisin)	Used for prevention and treatment of infections.
21	Trimethoprim	Used in treatment of urinary tract infections.
22	Tripelennamine (Pyribenzamine)	Used for treatment of cough and common cold.
23	Vitamin K	Used for healing of wounds and blood clotting.

1.4.2. Haemolytic sensitivity of Glucose-6-Phosphate Dehydrogenase variants to primaquine

Primaquine is the only medication currently available that can prevent *P. vivax* and *P. ovale* malaria from relapsing. This medication triggers a dose-dependent haemolysis in G6PD-deficient patients. Therefore, it is important to know the G6PD status of the individual prior to primaquine treatment. However, except few countries like Malaysia and Phillipines, this status is not tested when a person is diagnosed with *P. vivax* and *P. ovale* malaria. This puts the patient at risk for haemolysis when primaquine treatment is provided. Taking this into consideration, WHO has suggested the following recommendations for the safe administration of primaquine, which is necessary for the prevention of malaria caused by *P. vivax* and *P. ovale* (WHO, 2016):

i. Children and adults should receive a dose of 0.25–0.5mg base/kg body weight of primaquine for 14 days to prevent the recurrence of *P. vivax* and *P. ovale* malaria. However, this recommendation is not applicable in cases like expecting mothers, infants under the age of six months, nursing mothers and those with G6PD deficiency.
ii. A dose of 0.75mg base/kg body weight administered once a week in G6PD deficient

patients can be taken into consideration for preventing *P. vivax* and *P. ovale* malaria relapse. This treatment should be given for eight weeks and under close medical supervision.

iii. In cases where the G6PD status is unknown and G6PD testing is not available, the decision to prescribe primaquine should be made after weighing the risks and benefits of doing so.

iv. For women who are pregnant or nursing, weekly chaemoprophylaxis with chloroquine may be an option. Following delivery and the breastfeeding phase, primaquine treatment may be suggested to prevent recurrence of *P. vivax* and *P. ovale* malaria depending on the women's G6PD status.

1.5. Haemoglobinopathies

The word "haemoglobinopathy" serves as a comprehensive term for all genetically inherited haemoglobinopathies. Thalassaemia and structural haemoglobin variations are the two major categories for these disorders, both of which are brought about by mutations in the α- or β-globin genes of haemoglobin. While the structural variants of haemoglobin are caused by defects in the structure of any one of the subunits, thalassaemia is caused by a quantitative fault in the production of one of the globin subunits (Kohne, 2011). Both classes of disorders are by far the most commonly occurring monogenic disorders affecting a person's haemoglobin (Williams & Weatherall, 2012). The inheritance pattern of these disorders is autosomal recessive (Cao & Kan, 2013). Around 0.3–0.4 million children with such disorders are born each year in both low- and middle-income countries (Amjad *et al*., 2020). The Indian subcontinent, Southeast Asia, the Middle East, and Sub-Saharan Africa experience higher prevalence of these disorders (Weatherall, 2010).

1.5.1. Structural disorders of Haemoglobin

Amino acid substitution in any of the globin chains result in structural variations of haemoglobin (Hb), which might influence the stability or functionality of the affected haemoglobin. More than 700 structural variants have been identified, but only HbS, HbE

and HbC variants are widespread. The characteristic features of the common structural Hb disorders are presented in Table 4.

Table 4. Types of structural disorders of haemoglobin.

Haemoglobin S disorder	
i. Most dangerous haemoglobinopathy which causes RBCs to assume the shape of a sickle. ii. Sickling of RBCs is caused by polymerization of deoxygenated haemoglobin inside the RBC, making the RBCs to become fragile and sickle shaped. iii. Arises from substitution of Glutamine by Valine in the 6th residue of the β-globin subunit.	
Type	**Characteristics**
Haemoglobin S trait	i. A heterozygous condition where the affected individual inherits just one aberrant HbS allele. ii. Individuals do not show any symptoms of sickling, but are carriers of the abnormal HbS gene.
Haemoglobin S disease	i. Affected individual has homozygous HbSS inherited from affected parents. ii. Most severe form of HbS disorder. iii. Chronic haemolytic anaemia, pain crises, and a high susceptibility to infections are few of the symptoms that the affected person may experience. iv. Require medical attention and recurrent blood transfusions to survive.
Haemoglobin E disorder	
i. Caused by replacement of glutamate by lysine at the 26th residue of β-globin gene. ii. Mild anaemia and viral infections are associated with HbE, and certain drugs can cause haemolysis. iii. When combined with thalassaemias, HbE causes serious form of haemoglobinopathies.	

Type	Characteristics
Haemoglobin E trait	i. Heterozygous condition in which the patients act as carriers of the abnormal HbE. ii. Display variable hypochromic anaemia.
Haemoglobin E disease	i. Homozygous state in which the patients are associated with microcytic hypochromic anaemia ii. Haemolysis may occur due to some exogenous triggers.
Haemoglobin C disorder	
Glutamate is replaced by lysine in the 6th residue of the β-globin chain, to produce haemoglobin C (HbC).	
Type	**Characteristics**
Haemoglobin C trait	Heterozygous condition with normal phenotype.
Haemoglobin C disease	Homozygous HbCC disease may have chronic haemolytic anaemia (Karna *et al*., 2022).

1.5.2. Thalassaemia syndromes

Characterized by absence or very low buildup of any one globin subunit of haemoglobin, thalassaemia is of two types- α-thalassaemia and β-thalassaemia. Absence or low production of α-globin chain is called α-thalassaemia, whereas in β-thalassaemia, there is an absence or reduced production of β-globin subunit (Forget & Bunn, 2013). Table 5 summarizes the characteristics features of the different types of thalassaemia syndromes.

Table 5. Types of thalassaemia syndromes.

Type	Characteristics
α-thalassaemia	
α-thalassaemia minor	i. One of the α-globin genes is deleted. ii. Causes negligible protein deficiency. iii. Patients are carriers who exhibit no symptoms or indications of

	anaemia. iv. Does not require medication and the haemoglobin appear to be normal.
α-thalassaemia trait	i. Lack two α-globin genes. ii. RBCs are smaller than normal. iii. Asymptomatic but show mild anaemia. iv. Also called as mild α-thalassaemia.
α-thalassaemia intermedia	i. Deficient in three α-globin genes. ii. Characterized by severe anaemia. iii. Produces unusual haemoglobin called "haemoglobin H" which disrupts the RBCs' cell membrane and makes it ineffective at carrying oxygen (Harewood and Azevedo, 2023). iv. Develops severe anaemia and need blood transfusions to address it. v. Individuals do not live longer without treatment and usually die in their early teens (Camacho *et al*., 1999).
α-thalassaemia major	i. Complete deletion of all four α-globin genes resulting in synthesis of faulty haemoglobin called haemoglobin Bart's with four γ-globin chains. ii. Also known as "hydrops fetalis". iii. Individual do not survive or die shortly after birth (Lee *et al*., 2010). iv. However, if the condition is diagnosed in the uterus, which is very rare, they can be saved by blood transfusion at the time of pregnancy (Camacho *et al*., 1999).
β-thalassaemia	
β-thalassaemia minor	i. Arises due to fault in one of the β-globin genes. ii. Also known as β-thalassaemia trait. iii. Does not pose any significant problem in the normal haemoglobin

	function.
β-thalassaemia intermedia	i. Characterized by absence of two β-globin genes. ii. Cause severe anaemia and other grim health concerns such as shortness of breath, mild jaundice, enlarged spleen etc.
β-thalassaemia major	i. Most severe form of β-thalassaemia caused by complete absence of β-globin genes. ii. Develop extreme microcytic hypochromic anaemia, a condition characterized by RBCs smaller in size than the normal and have reduced red color. iii. Newborn child with this disorder appears normal at the time of birth, however after a few months the child develops several issues such as retarded growth, sporadic fever, slow bowel movements and many more. If untreated, the condition worsens and can lead to death as they enter into their late teenage. iv. Routine blood transfusion accompanied by extreme constant therapeutic care can be helpful in such patients.

1.6. Prevalence of haemoglobinopathies

According to World Health Organization, haemoglobinopathy affects 5.2% of the world's population (Modell & Darlison, 2008). The prevalence of haemoglobinopathies is higher in populations that are/were previously exposed to malaria, notably those caused by *P. falciparum* (Taylor *et al*., 2012a). The African, Western Pacific, Southeast Asian, and Eastern Mediterranean people have a very high prevalence of haemoglobinopathy carriers, but populations of American and European descent have a significantly lower frequency. It represents as one of the major health issues in the affected countries. HbS is widespread throughout the Sub-Saharan Africa, Mediterranean region, Middle East and the Indian subcontinent. A total of 40% of people worldwide are HbS carriers, and 20% are α-thalassemia carriers (WHO, 2021). Southeast Asia and the Mediterranean region have significant rates of α°-thalassaemia, a more severe form of α-thalassaemia (Hockham *et al*.,

2019). In some places of Southeast Asia, the carrier frequency of HbE, the most prevalent structural variant of haemoglobin, has risen to as much as 70% (Barrera-Rayes & Tejero, 2019). Due to the region-wide prevalence of β-thalassaemia, compound heterozygous HbE-β-thalassaemia is also common. While previously only seen in these areas, the disease has now spread to many other nations due to ongoing worldwide population migration (Williams & Weatherall, 2012).

1.7. Association of haemoglobinopathies with malaria

Individuals with haemoglobinopathies are thought to have a lower risk of malaria, although this depends on the kind of haemoglobin variant (Taylor *et al*., 2012b). It has been hypothesized that HbS, particularly in the heterozygous condition (HbAS), provides protection from both moderate and severe forms of malaria (Hill *et al*., 1991; Modiano *et al*., 2001; Mockenhaupt *et al*., 2004a). Additionally, it has been stated that the protective effect against malaria was increased in children with the Hb AS genotype from 20 to 56% (Gong *et al*., 2012). This protective effect of Hb S against a milder form of malaria has also been shown by a few genome-wide association studies (Malaria Genomic Epidemiology Network, 2014; Goheen *et al*., 2017). Both heterozygous and homozygous carriers of HbC have been shown to be protective against severe malaria, however the homozygotes were more significantly protected than the heterozygotes (Agarwal *et al*., 2000; Mockenhaupt *et al*., 2004b; Modiano *et al*., 2001; Taylor *et al*., 2012b). Although HbE is the most frequent structural form, very less is known about its malaria-protective effect. Heterozygotes with the HbAE genotype have some resistance to *P. falciparum* infection (Roberts & William, 2003; Chotivanich *et al*., 2002). There are quite a few studies that have illustrated this defense against severe malaria in people with α-thalassaemia (Mockenhaupt *et al*., 2004; Allen *et al*., 1997; Williams *et al*., 2005). The protective role of haemoglobinopathies against malaria has been validated in a number of ways, although additional research is still required to fully understand the protective processes (Williams & Weatherall, 2012).

1.8. Association of Glucose-6-Phosphate Dehydrogenase deficiency with haemoglobinopathies

There are conflicting viewpoints on the association between G6PD deficiency and haemoglobinopathies. G6PD deficiency and haemoglobinopathies are genetically independent disorders that assort independently (Balgir, 2008). Researchers have shown a markedly greater co-occurrence of G6PD deficiency and haemoglobinopathies in regions with high levels of both genes. A study with a small sample size revealed that HbS patients were more likely to have G6PD deficiency, although it had no impact on the patients' haematological indices (Awamy, 1992). In a study from the China-Myanmar border, co-inheritance of G6PD with HbE was reported in 23% of the study population (Deng *et al*., 2017). Additionally, a small percentage of Nepalese individuals with β-thalassaemia were found to have G6PD deficiency (Gautam *et al*., 2019). Some authors, on the other hand, do not support this co-relation. No difference in the prevalence of G6PD deficiency was observed between the sickle cell patients and normal population group (Bouanga *et al*., 1998).

1.9. Association of Glucose-6-Phosphate Dehydrogenase deficiency with malaria

Certain studies have postulated that G6PD deficient patients have resistance against malaria. The mechanism by which such patients are thought to be protected is decreased replication of the malaria parasite in the G6PD deficient RBCs. G6PD deficient RBCs have a shorter life span or are phagocytozed, due to which the malaria parasite is unable to complete its life cycle within the RBC. This observation of malaria protection, however, is not consistent across all G6PD defective genotypes. According to some studies, the probability of developing severe malaria was reduced by 58% in male hemizygotes and by 46% in female heterozygotes (Ruwende *et al*., 1995; Guindo *et al*., 2007). A different study revealed a similar reduction in males, but there was no reduction in the risk for female heterozygotes (Storey *et al*., 1979). However, some investigations have found that neither male hemizygotes nor female heterozygotes offer any protection against severe malaria (Baduom *et al*., 2019; Johnson *et al*., 2009).

1.10. Malaria in Northeast India

In India, various species of malaria parasites such as *Plasmodium malariae*, *P. vivax, P. ovale* and *P. falciparum* are prevalent, but only *P. falciparum* causes a malignant form of malaria. Malaria causes the infected red blood cells (RBCs) to stick to the walls of blood vessels that result in eventual destruction of the RBCs, thus compromising oxygen transport in the body. *Anopheles* mosquitoes are the vector agents responsible for transmission of these malarial parasites from one person to another (Menkin-Smith & Winders, 2023). The hot and humid environment of tropical regions serves as ideal conditions for mosquito breeding, thereby making the tropical regions more prone to malaria (Filho *et al*., 2019). Among the total malaria cases of the country, 15.2% were from the seven states of Northeast India, viz., Assam, Arunachal Pradesh, Meghalaya, Mizoram, Nagaland, Manipur and Tripura. *A. minimus* and *A. baimaii* are the two major malaria parasite carrying agents in this region (Sarma *et al*., 2019). A selection pressure is thought to have been applied by malaria to different forms of genes expressed in red blood cell polymorphisms, including α- or β-thalassaemia, G6PD deficiency, and haemoglobinopathies like sickle cell disease. When malaria infects a person with sickle cell haemoglobin, the cells that have been infected form sickle-shaped and are eliminated by the immune system. As a result, the sickle cell gene, ironically, aids in longer survival in those affected people, which is otherwise a potentially fatal disease (Balgir, 2008). In context to G6PD deficiency, although the specific process by which this deficiency protects against malaria is unknown, certain researchers have hypothesized that it might be because *P. vivax* is more susceptible to oxidative stress. Because G6PD deficiency increases oxidative stress in RBCs, this could negatively impact the parasite. Therefore, those who possess this mutation are somewhat protected from malaria (Moorthie, 2016).

1.11. Study area

Assam is a state in northeastern part of India with a land size of 78,438km^2. It shares its boundary with seven other Indian states, viz., Meghalaya, Mizoram, Manipur, Nagaland, Tripura, Arunachal Pradesh and West Bengal. It also shares its border with two

countries, Bangladesh and Bhutan. The total population of Assam (Census of India, 2011) is 31.205 million comprising a heterogenous population of diverse socio-cultural and ethnic makeup. Among the total population of India, 8.6% is constituted by the ethnic population groups, among which 3.7% is represented by the ethnic population of Assam (Census of India, 2011). Major ethnic groups of Assam are the Bodo, Karbi, Mishing, Sonowal Kachari, Rabha, Dimasa and the tea tribes. The tribal populations of Assam have their origin mainly from the Mongoloid and Proto-Australoid races. The Bodos, Rabhas, Garos, Sonowal Kacharis, Karbis, Mishings belong to the Mongoloid race, (Bordoloi *et al*., 1987) while the tea tribes belong to the Proto-Australoid race (Sarkar, 2021).

The sites selected in this study were Udalguri, Baksa, Chirang and Kokrajhar districts of Assam that cover the Indo-Bhutan transboundary region and constitute the Bodoland Territorial Region (BTR). BTR covers a total area of 9612 km^2 with a population of 3,151,047 as per 2011 census. Many communities, including Bodo, Assamese, Bengali, Koch-Rajbongshi, Rabha, Garo, Adivasi, Muslim, Nepali, etc., are inhabitants of the region. Around 35% of the total population of these districts is tribals. The Bodos make up the majority of the tribal population, followed by the Rabhas and Garos.

1.12. Rationale of the study

In areas like Assam, where malaria is an epidemic (Barman *et al*., 2023), G6PD deficiency seems to be very high. However, very little is known about the prevalence of G6PD deficiency in this region. G6PD deficient individuals are less likely to be affected with malaria. However, individuals with G6PD deficiency, if affected have a very high risk of haemolysis when administered with anti-malarials, specifically primaquine. Also, the extent of haemolysis in the deficient individual depends on the severity of the deficiency and the administered dose of primaquine. So, it becomes necessary to have accurate information on the G6PD status, severity of the deficiency (by identifying the causative mutation/variant) and appropriate dose of such drugs to be given to such patients. Prevalence pattern of the mutations in the region can also be used as genetic markers in population studies.

1.13. Objectives

The study was conducted with the following objectives:

i. Screening for haemogobinopathies and G6PD deficiency among the adolescents of tribal community of Kokrajhar, Chirang, Baksa and Udalguri districts of Assam.

ii. Molecular analysis of the G6PD deficient subjects.

iii. Screening of natural antioxidants as a pharmacological agent to alleviate the challenges associated with G6PD deficiency- a Molecular Docking-Simulation study.