Chapter – 5

DISCUSSION

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5.1. Prevalence of Glucose-6-phosphate Dehydrogenase deficiency

The incidence rate of Glucose-6-Phosphate Dehydrogenas (G6PD) deficiency fluctuates between 0 to 27% across various Indian communities, with an overall prevalence rate of 8% (Tripathy & Reddy, 2007; Kumar *et al*., 2016). Earlier, it was assumed that around 8% of the world's population carried the genes responsible for G6PD deficiency (WHO, 1989). Also, countries with a greater malaria frequency had a higher prevalence of the deficiency. However, recent ongoing global migration has significantly altered the demography of the population, causing pockets of population groups to acquire this condition. As a result, the deficiency has now spread to countries that were previously considered otherwise (Harcke *et al*., 2019). The distribution of G6PD deficiency frequencies throughout Indian states displayed a varied picture. A cross-sectional study involving study population from 15 different states of India showed that the deficiency is higher among the tribal population in comparison to the non-tribal population. This shows that tribal people who have the deficiency are the most susceptible to drug or infection induced haemolysis (Devendra *et al*., 2020). The tribal populations of Nagaland, Chhattisgarh, West Bengal, Dadra & Nagar Haveli, and Gujarat, have been reported with a greater frequency (>10%) of G6PD deficiency. However, in Tripura, Himachal Pradesh, Uttarakhand, Andhra Pradesh, and Madhya Pradesh, there have been reports of consistently low frequencies (<5%) of the deficiency (Mukherjee *et al*., 2015). An overall prevalence of 6.2% of G6PD deficiency was observed in the current study population of Sub-Himalayan region of Northeast India. In the Northeastern part of India, very few studies have been conducted on G6PD deficiency. All of the tribal groups from North-East India that were studied had G6PD deficiencies. The Angami Nagas (27.0%) from Nagaland had the highest frequency, followed by Rabhas (15.8%) and Mikirs (15.6%) from Assam (Bhatia & Rao,

1987). A hospital based study from different states of the region has reported 5.4% of G6PD deficiency (Bharti *et al*., 2019). Another hospital based study from the region has found a high prevalence (33.75%) of the deficiency among malarial patients (Rajkhowa *et al*., 2020). Comparatively, highest numbers of studies have been conducted among different tribal groups in the western states of India such as Maharashtra, Gujarat, Rajasthan, Dadra & Nagar Haveli, with prevalence of the deficiency ranging between 1.4-31.4% (Choubisa *et al*., 2009; Mukherjee *et al*., 2015).

5.2. Clinical presentation of Glucose-6-phosphate Dehydrogenase deficient subjects

Most of the time, G6PD deficient individuals are asymptomatic. However, G6PD deficiency may cause neonatal jaundice, haemolytic anaemia, favism following consumption of broad beans, and cause oxidative haemolysis which, occasionally, can result in severe haemolytic anaemia following treatment with specific drugs or precipitated by specific infection (Luzzatto *et al*., 2020, Elsea *et al*., 2023). In the present study, all the deficient cases were asymptomatic, except a few having neonatal jaundice. In some countries such as Malaysia, where G6PD deficiency is commonly seen, it turns out to be a major contributing factor to severe neonatal jaundice (Lee *et al*., 2022). Exposure of a G6PD deficient neonate to oxidative chemicals in the environment, such as napthalene can also result in severe neonatal jaundice from acute haemolysis (Santucci and Shah, 2000; Volney *et al*., 2018). G6PD deficient neonates has also been documented to develop jaundice from nursing mothers consuming broad beans and using naphthalene balls (Lee *et al*., 2022) Although not all G6PD deficient neonates experience neonatal jaundice, multiple studies conducted worldwide have consistently shown that the frequency of neonatal jaundice in G6PD deficient babies is higher than that of G6PD normal controls (Olusanya *et al*., 2014; Altay & Gumruk, 2008; Haloui *et al*., 2016; Elella *et al*., 2017).

5.3. Co-relation of Glucose-6-phosphate Dehydrogenase deficiency with haematological indices

In the present study, the RBC parameters such as Hemoglobin (Hb) concentration, Red blood cells (RBCs) counts and Mean Corpuscular Haemoglobin Concentration (MCHC) appeared to be significant in G6PD deficient cases. These observations of co-relation of Hb and RBC with G6PD are in agreement to the studies of Jarullah *et al*., 2015. The other RBC parameters, MCV, MCH, PCV, RDW and platelets did not show statistically significant co-relation. Similar observation of haemotological parameters were reported in G6PD deficient patients with malaria, wherein no significant differences were observed between G6PD and the haematological parameters MCV, MCH, PCV, RDW and platelet counts (Kotepui *et al*., 2016). With the aging of RBCs, their natural enzymatic activity decline, making them more vulnerable to haemolytic risk. However, in healthy people, the RBCs have a substantial excess of potential G6PD activity, which enables the PPP to be markedly up-regulated in response to oxidative stress (Salvador & Savageau, 2003). Consequently, the natural process of enzyme degradation does not proceed to the point where the person is clinically at risk; but, in G6PD deficient individuals, it results in haemolytic crises.

Significant higher monocyte counts were reported in G6PD deficient individuals with malaria compared to the individuals having normal G6PD level (Kotepui *et al*., 2016). The reason for presence of high monocytes in G6PD deficient patients may be explained by the observations of earlier workers who reported the presence of hemozoin in certain portion of circulating phagocytic cells which is substantially linked with parasitemia and disease severity in malaria patients (Metzger *et al*., 1995; Nguyen *et al*., 1995). The inability of hemozoin-loaded monocytes to repeat the phagocytic process and to produce an oxidative burst renders them functionally compromised as well (Schwarzer & Arese, 1996). Furthermore, when the parasites induce oxidative stress, G6PD-deficient red blood cells will lyse and send out signals to be phagocytosed more frequently than normal red blood cells. This may be the reason why people with a G6PD deficiency only have mild to moderate malaria rather than severe malaria (Kotepui *et al*., 2016). Although statistically not significant, a slight increase in the monocyte counts of the G6PD deficient subjects were recorded in the present study. A study has reported a positive co-relation between

leucocyte count and G6PD in people with normal G6PD level, suggesting that the leucocytes could be a significant factor in G6PD level (Ajlaan *et al*., 2000). Leucocyte and other WBC parameters such as lymphocytes and eosinophils were a little higher in the deficient subjects; however, they were not significant. This may be due to the reason that the deficient subjects in the study did not have malaria or any other infection at the time of their blood sample collection.

5.4. Association between Glucose-6-phosphate Dehydrogenase deficiency and gender

Being an X-linked disease, males are more likely to be affected with G6PD deficiency. In the present study too, higher cases of the deficiency were recorded in male subjects compared to females. Similar higher incidences of the deficiency were reported in male study subjects (Chinevere *et al*., 2006; Khim *et al*., 2013; Kotepui *et al*., 2016). A male can only be hemizygous G6PD normal or hemizygous G6PD deficient because he has only one *g6pd* allele; a female, on the other hand, can be homozygous G6PD normal, homozygous G6PD deficient, or heterozygous for G6PD deficiency due to the presence of two *g6pd* alleles. Again, according to population genetics principles, heterozygotes are more common than hemizygous G6PD-deficient males in any given group, and homozygous G6PD deficient females are far more rare (Luzzatto *et al.*, 2016). In the present study population, 68.7% of the total deficiency was seen in males and 31.3% in females.

5.5. Haemoglobinopathies

Globally, haemoglobinpathies such as thalassaemias, HbS, HbE, HbC, etc., are the most prevalent hereditary diseases, turning into a major disease burden in the affected countries. Every year, about 3, 00,000 - 5, 00,000 neonates with Hb disorder are born, and around 7% of people of the world population are thought to be carriers of abnormal haemoglobin gene (Weatherall, 2001). An overall prevalence of 42.7% of haemoglobinopathies has been recorded in the present study. Among the tribal population

of India, haemoglobinopathies, especially those involving HbS, HbE and β-thalassaemia, pose significant issues. The tribal population in India, constituting about 8% of the country's total population, comprises of a heterogenous mixture of population having distinctive genetic identities. Over the centuries, tribal people have been living in isolation and practicing endogamy, which has facilitated in maintaining distinct genetic characteristics. Based on their genetic identity, the Indian tribal population is broadly classified as tribal populations in the Northeast, the Tea Garden tribes, the Central Indian tribes, the Western Indian tribes, the Eastern Orissa and Andhra Pradesh pribal populations, and the Southern Indian tribal populations (Ghosh *et al*., 2015). β-thalassaemia carriers account for 3-4% of the total population, with higher prevalence in communities like Punjabis, Bengalis, Gujaratis, Sindhis, Lohanas, etc. (Sinha *et al*., 2009; Madan *et al*., 2010; Mohanty *et al*., 2013).

The majority of tribal populations in the Northeast region (Mongoloids) have HbE, which also occurs in combination with β-thalassaemia and α-thalassaemia genes. This variant of Hb is the most common haemoglobinopathy in the Southeast Asian countries and Northeast India as well. In the Northeastern states, the carrier frequency of HbE is as high as 50% in some areas (NHM, 2016). A very high prevalence of HbE (64.5%) was reported among the Bodo-Kachari and other Tibeto-Burman linguistic groups of Assam (Deka *et al*., 1988). In the present study, 35.2% of the anaemic cases were found to have HbE disorder, with 23.9% as carriers and 11.4% as disease cases. A study from Manipur has reported a prevalence of 10.53% of HbE carriers and 1.05% of HbE disease among four endogamous populations (Singh *et al*., 2010). While, the most common haemoglobinopathy among the tribal people that work in the tea gardens of Northeast India (Proto-Australoids) is HbS (Ghosh *et al*., 2015). The presence of high HbS has also been supported by recent studies wherein, a prevalence of 11.06% of HbS has been recorded among children of Udalguri district. Among these, 6.4% were HbS carriers and 4.7% were HbS disease cases (Babu *et al*., 2021). The present study observed a higher prevalence of HbS carriers (29%) and 3.9% of HbS disease among the Proto-Australoid population. Such high prevalence of HbS may be attributed to the fact that the tea tribe populations (Proto-Australoid) in Northeast India

were the descendents of the tribal population in Central India and parts of Orissa and Jharkhand, where HbS has been found to be the predominant Hb disorder.

Compound heterozygosity for HbE and HbS is a rarely observed condition with about 46 cases being reported worldwide. This is a clinically silent condition compared to other forms of haemoglobinopathies (Gurkan, 2006). Two cases of compound heterozygous condition for HbS and HbE were found in the present study. Both cases were from the tea tribe community. This may have resulted from intermarriages between the communities having high prevalence of HbE and HbS.

High numbers of cases (86) with hereditary persistence of foetal Hb (HbF) were observed in the study. In sickle cell disease patients, it typically takes five to ten years for HbF to transform to adult haemoglobin, which is hardly completed (Steinberg, 2020). In the present study, presence of high HbF may account for the lower Hb A_0 in HbS disease patients.

5.6. Association between Glucose-6-phosphate Dehydrogenase deficieny and haemoglobinopathies

Co-occurrence of G6PD deficiency and haemoglobinopathies has conflicting views as reported by many workers. In the study, only one case was detected with both the conditions. This may be supported by the view that G6PD deficiency and haemoglobinopathies are genetically independent disorders that assort independently (Balgir, 2008). The case was detected in a female (52 years) of Mongoloid race, with HbE disease and G6PD A^+. Even though the case subject had both G6PD A^+ variant and HbEE, the subject did not show any clinical complications or have no record of haemolytic crisis. Except this, none of the cases out of 2310 were detected with co-occurrence of the two conditions.

5.7. Glucose-6-phosphate Dehydrogenase variants

Extensive investigations on the prevalence of G6PD deficiency in various population groups has been performed, but studies on the molecular basis of the deficiency in Indian population are still lacking. Such information on the molecular basis of the disease is important to have an understanding of the risks linked with exposure to various oxidative triggers. These triggers cause varied degrees of haemolysis depending on the severity of the deficiency. The more severe the deficiency, the more severe is the haemolysis. Among different oxidative triggering agents, primaquine-induced haemolysis is the most harmful. When people with this deficiency are treated with primaquine, they experience a dose-dependent haemolysis. Taking this into account, it is critical to understand the G6PD status and the severity of the deficiency in deficient patients. According to the WHO categorization (WHO, 1989), the variants classified as class I and II induce a more severe form of the deficiency with enzyme activity of <10%. Class I variants are associated with chronic non-spherocytic hemolytic anaemia (CNSHA) and are sporadic in distribution. The common biochemical characteristics shared by these variants included a low inhibition constant (Ki) for NADPH, poor thermostability, and an enhanced Michaelis constant (Km) for substrates. These variants were predominantly found in *g6pd* gene mutations affecting exon 10 (Fiorelli *et al*., 2000; Bancone *et al*., 2021). Class II and III variants have very little diversity in their residual enzyme activity but a lot of variance in their protein characteristics. Thus, this categorization which is based on the enzymatic activity alone is not sufficient to distinguish between the two classes (Luzzatto & Poggi, 2009).

Mutations in the *g6pd* gene induce varying degrees of enzymatic activity, reducing the gene's ability to cope with oxidative challenges caused by various triggers. The most prevalent mutation to date among those known to exist is G6PD Mediterranean. Based on its enzymatic and biochemical characteristics, this variant is classified as a class II variant. Severe neonatal jaundice is thought to be associated with this variant (Ezz El-Deen *et al*., 2013). On exposure to certain triggers such as drugs, infection or fava beans, individuals with this variant are considered to develop acute haemolytic anaemia (Capellini and Fiorelli, 2008). This variant is observed in all the countries that encircle the Mediterranean

Sea (Luzzatto *et al*., 2001). In different populations of India too, this is the most commonly detected variant. In the present study, however, only 8.3% (n=12) of the total deficient cases belonging to both Proto-Australoid and Mongoloid population carried this variant. Out of 12 samples found to have this mutation, two were from the Proto-Australoid population and 10 from the Mongoloid population.

The second most common variant in Indian population is G6PD Orissa. This variant caused by Ala→Gly mutation at the 44th codon of exon 3 is classified as a class III variant. Mutations of this class have a lowered enzyme activity between 10-60% and acute haemolytic anaemia may develop when exposed to certain oxidative triggers. The tribal population of Orissa and Madhya Pradesh as well as other tribal and caste groups of India has been reported to harbor this mutation (Kaeda *et al*., 1995; Sukumar *et al*., 2004). These two variants, i.e., Mediterranean and Orissa accounts for about 80.1% of the total G6PD deficiency in India (Devendra *et al*., 2020). In the present study, G6PD Orissa was found to be the most common variant accounting to about 63.9% (n=92) of the total deficiency. The Proto-Australoid population group only was seen to carry this mutation. Such high prevalence of the variant among the Proto-Australoids may be due the fact that they had their ancestral origin from the tribal groups of states like Jharkhand, Chattisgarh and Orissa, where the variant is common.

The other commonly encountered variant in India is the G6PD Kalyan-Kerala/Jamnagar/Rohini. Earlier the two variants Kalyan and Kerala were considered different based on their biochemical properties. Both the variants showed mild deficiency. Later, molecular analysis revealed that both the variants result from a same mutation Glu→Lys at 317th codon of exon 9. This mutation was then renamed as G6PD Kalyan-Kerala. Again, based on biochemical characterization, two other variants Jamnagar and Rohini were also thought to be distinct variants (Sayyed *et al*., 1992; Sayyed *et al*., 1994). When analyzed at the molecular level, these two variants also showed the same mutation as that of Kalyan-Kerala (Sukumar *et al*., 2005). This mutation was observed in 9.02% (n=13) of the total deficient cases from both the Proto-Australoids (n=2) and the Mongoloids (n=11).

Although the above discussed variants, viz., Mediterranean, Orissa and Kalyan-Kerala are the most common variant in India, these variants have not been reported earlier from the tribal populations of the Northeastern part of India. These three variants account for 81.25% of the total deficient cases of the study. Two other variants, G6PD A^+ and Mahidol were also detected in the study. The variant G6PD A^+ (126 Asn→Asp), a class III-IV variant was detected in 11.8% (n=17) samples. Among 17 samples, one was from the Proto-Australoid population and 16 were Mongoloids. This variant was also reported in three individuals from Northeast India in a hospital-based study. Out of these, one was found to have the additional 202 G>A (A^{-202}) mutation (Rajkhowa *et al*., 2020). In our study, this additional mutation, however, was not found in any of the 17 samples with A^+. Mahidol variant was detected in only 1.38% of the samples who had their origin from the Mongoloid race. A recent study from four hospitals of Northeastern region of India has stated that 88.9% of the study samples had this variant which is caused by the mutation 199 Ile→Val (Bharti *et al.*, 2019). This variant is one of the most common variant in the Southeast Asian population.

In the study, eight samples remained uncharacterized as the mutations could not be identified by any of the restriction endonucleases used. This may be due to the reason exons 1, 2 and 8 were not included in the study, mutations present in these exons (if any) could not be identified. Thus it is obvious that other mutations also exist among the study population as well as other groups of population in the region.

5.8. *In- silico* study

Over 60 years have passed since the discovery of G6PD-associated enzymopathy; however, no treatment has been developed yet for the deficiency. The most severe form of G6PD deficiency being rare and the common Class II and III variants shows symptoms of the deficiency only on exposure to oxidative triggers, the development of a treatment for this deficiency is of little interest to pharmaceutical companies. Furthermore, till date there are large numbers of mutations causing the deficiency and hence the consequence of these large numbers of mutations on protein structure is diverse. As a result the possibility of

developing ‘one-size fits all’ therapy seems uncertain for this deficiency (Garcia *et al*., 2021). There are some remedial prospects for G6PD deficiency that can be explored, such as antioxidant therapy, improving glutathione biosynthesis via N-acetyl-cysteine (NAC) administration, generation of NADPH via complementary pathways, G6PD upregulation via transcriptional regulators, small molecules that correct enzyme dysfunction by directly binding to G6PD, small molecules that increase the transcriptional output of G6PD and gene therapy. However, all of the above have not been completely successful in developing a therapy for the disease. In the present study, an attempt was made to assess the efficacy of the naturally available antioxidants to overcome G6PD deficiency associated challenges in four variants, viz., Orissa, Kalyan-Kerala, Mahidol and A^+ using *in-silico* methods such as molecular docking (structure-based docking) and molecular dynamics simulation.

The cytoprotective properties of natural antioxidants against oxidative stress and the various defensive systems involved are of great interest nowadays. Several natural antioxidants have been identified from different medicinal plants. The ability of these compounds to tackle oxidative stress has broadened its use in treatment of diseases relating to oxidative stress (Mehta *et al*., 2018). In G6PD deficiency, insufficiency of NADPH production causes an increased oxidative stress. Till date, only a few antioxidants such as Vitamin C, Vitamin E, astaxanthin and α-Lipoic acid has been studied for treatment of G6PD deficiency (Garcia *et al*., 2021). Thus, in the present study, the efficacy of these natural antioxidants in minimizing the challenges associated with the deficiency has been studied through an *in-silico* approach.

The key to successful structure-based molecular docking is identification of the binding site (Ferreira *et al*., 2015). In many cases, the binding site (binding pocket) is known; but in some cases there are no available information regarding the binding site for a target, or, a putative binding site may have been identified by computational or experimental methods, but without any information on the druggability of the target. Therefore, in these situations, it is critical to comprehend druggability in order to steer clear of unachievable goals and concentrate drug research efforts on locations with better prospects (Schmidtke and Barril, 2010). Numerous binding sites are present in the refined

G6PD, including a substrate-binding site, two NADP binding sites, and a dimer interface binding site. In the present molecular docking study, the binding pockets were identified and binding affinities of the antioxidant compounds with G6PD Orissa, Kalyan-Kerala, Mahidol and A^+ were explored, where it was seen to be ranging between -5.2 to -9.2kcal/mol, -5.1 to -9.8kcal/mol, -5.2 to -9.4kcal/mol and -5.0 to -10.5 kcal/mol respectively. Mutations in the *g6pd* gene reduce the efficiency of the monomers to dimerize, leading to a reduction in binding energy required for homodimer formation (van Wijk *et al*., 2004). In the current docking study, although not all compounds showed good binding affinity, the average binding affinities of the compounds were observed to be -7.5kcal/mol, -7.15kcal/mol, -7.07kcal/mol and -7.05kcal/mol with Orissa, Kalyan-Kerala, Mahidol and A^+ respectively. *In-vitro* and *in-vivo* studies on efficacy of three antioxidant compounds, namely, astaxanthin, vitamin C and vitamin E have been conducted earlier, but were not completely successful in minimizing G6PD related complications (Garcia *et al*., 2021). This result is supported in the current molecular docking study too, as astaxanthin, vitamin C and vitamin E showed comparatively lower binding affinities with the four G6PD variants. Following docking, drug-likeness and toxicity analyses were performed.

For early-stage drug research, the idea of drug-likeness offers helpful suggestions by assessing certain physico-chemical parameters (Ursu *et al*., 2011). Prior to moving forward with experimental trials, drug discovery pipelines frequently use *in-silico* toxicity screens to examine the pharmacokinetic and pharmacodynamic features of the chosen drug-like molecules (Ntie-Kang, 2013). The most popular way to judge drug-likeness is through rules, the most well-known of which is Lipinski's Rule of Five (Lipinski *et al*., 2001). The rule indicates that when two or more of the following physico-chemical conditions are violated, a substance is more likely to show poor absorption or permeation: more than five hydrogen-bond donors or more than ten hydrogen-bond acceptors (nitrogen and oxygen atoms), molecular weight (MW) is larger than 500 Da and the computed logP (C log P) is greater than 5. In the current study, eight compounds, namely, Alpha-carotene, Astaxanthin, Beta-Carotene, Cantaxanthin, Chicoric acid, Lutein, Theaflavin and Zeaxanthin did not have the drug-likeness properties with 2-3 violations of Lipinski’s rule,

although they showed good binding affinities with the variants. However, it is reported that 6% of oral medications don't meet two or more requirements, and 16% don't meet at least one of the Lipinski's Rule of Five. As for instance, the well-known drugs like Atorvastatin and Montelukast violate one of the Lipinski rules (Bickerton *et al*., 2012). Again, toxicity analysis is also a crucial step in drug designing to determine the possible negative consequences that the substances may have. The LD50 values (mg/kg body weight), that is the median lethal dose at which 50% death of test subjects occur when exposed to a compound gives an indication of the toxicity of the substances. Based on this LD50 values, compounds are classified as toxic or non-toxic. Out of all antioxidants considered in the study, Catechin, Canthaxanthin, Epicatechin, Epigallocatechin, Gallocatechin and Lycopene were only found to be non-toxic.

Based on the overall observations, four complexes were selected and analyzed using LigPlot+, viz., Orissa-Myricetin, Kalyan-Kerala-Apigenin, Mahidol-Catechin and A^+-Diadzen. The two types of interactions, hydrogen and hydrophobic were analyzed using LigPlot+, as these are considered to play a decisive role in molecular docking. The docking mechanism and binding mode selection are most likely significantly influenced by this hydrogen bond (Fikrika *et al*., 2016).

To further validate or to ascertain the complexes's stability, molecular dynamics simulation of the docked complexes was performed. For accurate drug binding prediction as well as prediction of associated thermodynamic and kinetic parameters, receptor and ligand flexibility is essential (Fischer *et al*., 2014). Molecular dynamics simulation offers a picture of the structural flexibility by analyzing the physical movements of atoms and molecules of a ligand-target model system (Durrant & McCammon, 2011). To assess the stability of the ligands and variant C-α atoms in the ligand-variant complexes over time, the molecular dynamics production trajectories were superimposed on the initial structures to calculate the RMSD of these structures. Comparison of the four variants of G6PD detected in the study with the WT G6PD revealed that Kalyan-Kerala and A^+ showed larger deviation compared to the WT protein. Decrease in the stability of these variants may be supported by the observation that mutation lying in the functional region of the enzyme

distorts the functionality and stability of the enzyme (Cunningham *et al*., 2017). G6PD Orissa was seen to have similar RMSD to that of the WT protein, indicating that the stability is similar in both.

Again, among the four complexes of the study, Kalyan-Kerala-Apigenin was observed to have the least deviation compared to the WT G6PD. The other complexes, namely, Orissa-Myricetin, Mahidol-Catechin and A^+-Daidzen did not improve the stability of the variants. This may be due to the fact that only one compound, namely, Myricetin, Catechin and Daidzen were selected for Orissa, Mahidol and A^+ respectively, were selected for simulation study. Molecular dynamics simulation of larger number of complexes with inclusion of ligands with one or two violations of Lipinski's rule could have provided better information on the efficacy of the antioxidants in these variants. Thus, it may be summarized that even though the selected ligands did not violate any of the Lipinski's rule, molecular dynamics simulation did not support the efficacy of these three compounds in improving the conditions of the three variants, Orissa, Mahidol and A^+.

5.9. Significant findings of the study

The extent of haemolysis in the G6PD deficient people depends on the severity of the enzyme, which can be known upon identification of the causative mutation/variant. Thus it is important to understand the G6PD status prior to providing antimalarial treatment in areas where malaria is high. The present study recorded an overall 6.2% (n=144) prevalence of G6PD deficiency among the study population. Significant positive co-relation of G6PD was observed with Hb, RBC and MCHC. PCR-RFLP analysis showed the presence of five mutations in the study population, viz., Orissa (44 Ala→Gly in exon 3), A^+ (126 Asn→Asp in exon 5), Mahidol (163 Gly→ Ser in exon 6), Mediterranean (188 Ser→Phe in exon 6 and Kalyan-Kerala (317 Glu→Lys in exon 9). Among these, only one variant, G6PD Mediterranean was a class II variant as per WHO classification. The other four variants were class III variants. Although G6PD Meditteranean is the most common variant in Indian population, followed by Orissa and Kalyan-Kerala, these variants were, however, not reported earlier from the tribal population of Northeastern region of India.

These three variants were detected in the present study, with G6PD Orissa as the most common variant. The variant was found exclusively in the Proto-Australoid population; G6PD Mediterranean, Kalyan-Kerala along with another variant A^+ was observed in both the Proto-Australoid and Mongoloid populations. The other variant G6PD Mahidol was seen in Mongoloid population.

A significantly high prevalence of 42.7% of haemoglobinopathies was also observed in the study, with 210 as HbS carriers and 53 as HbS disease, 114 as HbE carriers and 109 as HbE disease. Although HbE is generally not seen in Proto-Australoid, HbE cases were found four Proto-Australoids in the study. Additionally, two samples from the Proto-Australoid population were found to be compound heterozygous for HbS and HbE, which is generally a very rarely encountered condition. Hb E-β thalassemia trait was discovered in another Proto-Australoid sample. Except one from Mongoloid population, none of the samples were found to have both G6PD deficiency and haemoglobinopathies. High HbF values were observed in 86 numbers of HbE and HbS disease cases.

Till date, no treatment has been developed for the treatment of G6PD deficiency. Few remedial prospects for treatment of the deficiency have been explored, but, they have not been completely successful in developing a therapy for the disease. The reason for this may be due to the large numbers of mutations causing the deficiency resulting in diverse effects on the protein structure. Thus, an attempt to assess the efficacy of the naturally available antioxidants as a pharmacological agent to overcome G6PD deficiency associated challenges in the four detected variants, viz., Orissa, Kalyan-Kerala, Mahidol and A^+ has been made using *in-silico* methods. The binding affinity, drug-likeness and toxicity of the natural antioxidants were analyzed. These analyses showed the best fit between Orissa and Myricetin, Kalyan-Kerala and Apigenin, Mahidol and Catechin and A^+ and Daidzen. Among these complexes, Kalyan-Kerala-Apigenin showed an improved stability compared to the mutant.

5.10. Future scope of the study

Due to limited resources including financial, logistics and time constraints, there are certain limitations in the study, which can be carried forward to future studies. The study was conducted only among the tribal population (n=2310) of the region. Thus, the reported prevalence of the deficiency is based on the study population only, which may not reflect the actual prevalence in the region. A larger study focusing on the tribal population as well as other communities of the region can be conducted to have an enhanced understanding on the G6PD status of the region. The commonly reported mutations in India and the neighbouring countries (exons 3, 4, 5, 6, 7, 9, 10, 11 and 12) were taken into account in the study. Examination of other mutations also seems to be important as eight samples in the study remained uncharacterized. Moreover, molecular docking showed good binding affinities with many antioxidants, but based on other parameters such as drug-likeness and toxicity, only one complex was selected for molecular dynamics simulation, due to which expected results could not be obtained. Molecular dynamics simulation of all the antioxidant-variant complexes will give a better insight on the efficacy of the antioxidants to treat G6PD deficiency in the selected variants.