



Glucose -6-Dehydrogenase Deficiency and Malaria

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REVIEW

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most prevalent enzyme disorder, estimated to affect 400 million people worldwide. Mutations in the G6PD gene, situated in the long arm of the X chromosome, result in different G6PD deficiency variants, producing a wide range of biochemical and clinical phenotypes, which can be further distinguished by differences at the molecular levels. Drug related haemolytic anaemia was first observed with the use of Primaquine, and was subsequently discovered to be caused by G6PD deficiency. The link between G6PD deficiency and malaria is further bolstered by the observation of the global distribution of G6PD deficiency being parallel with that of malaria. This observation led to the suggestion that G6PD deficiency has protective advantage as a defence against malaria. Different G6PD deficiency variants have different propensities to cause haemolytic anaemia on exposure to oxidant drugs. Knowledge of the different biochemical and molecular variants of G6PD deficiency is important not only between people in different continents but even between people in different regions of the same country. G6PD deficiency produces a wide spectrum of clinical disorders, whose observable differences can be due to different oxidant drugs, different infections, and even different age groups.

Key Words

Glucose 6 Dehydrogenase Deficiency, malaria, drug induced haemolysis

The global distribution of glucose-6-phosphate dehydrogenase (G6PD) deficiency closely follows the world-wide distribution

of malaria. It has been suggested by Beutler¹ that although malaria is a disease of high morbidity and mortality, it may, nevertheless, contribute to the balanced polymorphism and relatively high frequency of G6PD deficiency, because of the latter's protective advantage as a defence against malaria. For this reason, and because the first observed incidence of drug related haemolysis occurred after the administration of the anti-malarial drug primaquine, many studies on G6PD deficiency are related to malaria. Although any one of a large number of oxidant drugs will cause haemolysis in G6PD deficient patients, primaquine, because of its wide usage in malaria treatment, remains an important cause of haemolytic anaemia in clinical practice. G6PD is required in the production from nicotinamide adenine dinucleotide phosphate (NADP) of NADPH, which is needed to maintain glutathione in the reduced state to prevent haemolysis from oxidant stresses.

Primaquine, an early anti-malarial drug, is still important, despite numerous recent additions to the list of anti-malarial drugs, because of its unique property of being able to kill both schizonts and hypnozoites in the hepatic stage of the life cycle of the malaria parasite.² In regions where both the prevalence of malaria and the prevalence of G6PD deficiency are high, haemolytic anaemia, consequent upon primaquine usage in the eradication of malaria, becomes an important issue.³ First, the prevalence of G6PD deficiency and the types of G6PD variants in their global distribution will be reviewed. Second, the malaria parasite life cycle with special reference to the place of primaquine in the eradication treatment of malaria will be discussed. Third, the broad clinical spectrum of G6PD deficiency will be considered.

Prevalence of G6PD deficiency, and types of variants

G6PD deficiency is the most prevalent enzyme disorder in the world, and is caused by mutations in the G6PD gene, which is situated in the long arm of the X chromosome, close to the gene for haemophilia A and colour blindness. Most of the gene mutations are single base substitutions, resulting in amino acid replacements. Gene mutations result in protein variants with different levels of enzyme activity, producing a wide range of biochemical and



clinical phenotypes.⁴ The inheritance of the disorder is X linked and recessive in type and variably expressed in heterozygous females.

Diagnosis of G6PD deficiency is based on the biochemical estimation of residual enzyme activity, and electrophoretic mobility. The variable stability of the enzyme under different transport conditions and the variations between different enzyme estimation methods, give rise to heterogeneity in regional and global prevalence. Over twenty years ago, it was estimated that over 400 million people world-wide are affected by G6PD deficiency.⁵ Recently on the basis of meta-analysis of 280 prevalence estimates from 88 different countries, the global prevalence of G6PD deficiency was estimated as 4.9%. A wide range of variations was reported regarding G6PD enzyme activity among heterozygous females. i.e. only 14% of heterozygous females had deficient G6PD activity, while 33.3% had intermediate activity, and over 50% had normal activity.⁶ The prevalence of the most common G6PD variants in regions where antimalarials were used, was reviewed by Beutler.⁷

In Sabah, Malaysia a study of 1103 ethnic patients, Kadazan, Chinese and Bajau, the prevalence of G6PD deficiency was 9.8%.⁸ Lie-Injo found a prevalence of G6PD deficiency of 6.8% among 1008 subjects of more than 9 ethnic origins from three states of Sabah, Brunei and Sarawak. Of 485 Malays examined, the prevalence was 7.0% and of 165 Kadazans examined, the prevalence was 12.1%. The prevalence of G6PD deficiency in Malays varied from 1.1% in Djakarta in Indonesia, 0.6 to 2.0% in Singapore to 12.7% in the Philippines.⁹ Heterogeneity in prevalence was illustrated within regions and also within ethnic groups in different regions. DNA analysis undertaken in south west China revealed two common mutations at G1388A and G1376T, and the incidence of G6PD mutation is much higher in south west China than in other parts of the country.¹⁰

In Africa, the prevalence of G6PD deficiency varied from 28.0% in Nigeria to 3.6% in Comoros Island.⁷ Biochemical analysis shows, two types of mutations are common in Africa, G6PD A and G6PD A-. G6PD A mutation produces enzyme with normal activity and is electrophoretically rapid. G6PD A- has the same rapid mobility, but has only 10% of normal activity. DNA analysis shows G6PD A has a single substitution of aspartic acid for asparagine. The same mutation occurs in G6PD A-, but the enzyme deficiency is due to a second amino acid substitution, at one of three locations. Thus at the biochemical level, there is but one G6PD A-, but at the molecular level, there are 3 types.¹¹

G6PD Deficiency and Malaria Treatment Antimalarial agents and drug induced haemolysis

Antimalarial agents such as chloroquine, mefloquine, and doxycycline, do not have effect on the initial phase of malaria parasites within the liver, rather they act later in the life cycle of malaria parasites, after their release into the blood stream from the maturation phase in the liver. Blood stage schizonticides such as chloroquine, doxycycline, mefloquine, and atovaquone-proguanil interrupt multi-nucleated schizont development within erythrocytes, thus preventing clinical manifestations of malaria infection.

On the other hand, hepatic-stage schizonticides such as atovaquone-proguanil and primaquine destroy malaria parasites during active development within hepatocytes, killing liver schizonts of all four species of malaria parasites. However, atovaquone-proguanil does not act on hypnozoites in the liver, which exist in *P. vivax* and *P. ovale* infection. In order to achieve radical cure and prevent relapses of clinical malaria, it is important that the hypnozoites in the liver are destroyed. Primaquine is the only drug capable of killing the quiescent hypnozoites, thus preventing late onset clinical relapses of malaria. However, the use of primaquine for the effective prevention of clinical relapses in *P. vivax* and *P. ovale* infections has to be cautious due to its ability to cause haemolysis in G6PD deficient patients.²

The Clinical Spectrum of G6PD Deficiency

(a) G6PD deficiency related haemolysis: the historical background

Haemolytic anaemia occurring after the administration of antimalarial drug primaquine was reported as early as 1926. However, the discovery that the mechanism of haemolysis is due to G6PD deficiency was not possible until after the biochemical pathways of glucose metabolism have been discovered. In the Embden-Meyerhof pathway, glucose is converted to lactate with the production of ATP. In the alternate pentose phosphate pathway, the 6-carbon glucose is converted to the 5-carbon ribulose with the production of NADPH from NADP. The production of NADPH is G6PD dependent.

Beutler suggested that the clinical investigations into the mechanism of primaquine-induced haemolysis could not have been possible without the participation of prisoner volunteers serving sentences in the Illinois State Penitentiary at Joliet. It was noted that when human



volunteers were given primaquine, some developed acute haemolytic anaemia while most did not. When labeled erythrocytes from primaquine sensitive subject were transfused into normal subject, primaquine administration caused destruction of labeled cells but not the host cells. On the other hand, when labeled cells from normal subject were transfused into primaquine sensitive subject, primaquine administration caused destruction of host erythrocytes but not the labeled erythrocytes. The defect was thus proved to be an intrinsic erythrocyte defect.¹

Subsequent study on the cause of primaquine induced haemolysis led to the discovery that erythrocytes from primaquine sensitive individuals were unable to maintain glutathione levels after oxidative challenge. Further study on the maintenance of glutathione in the reduced state led to our present understanding that primaquine induced haemolysis is due to G6PD deficiency. The interaction of drug with erythrocyte produces hydrogen peroxide, which is damaging to the cell. Glutathione removes the peroxide, when the oxidized glutathione disulfide is converted to the reduced form by the enzyme glutathione reductase with the help of the co-enzyme NADPH, the production of which is G6PD dependent. The inadequate removal of peroxide causes the formation of denatured haemoglobin, which on binding to the cell membrane becomes visible as Heinz bodies. During passage through the spleen, Heinz bodies of the erythrocytes are removed with a portion of the cell membrane. After several passages through the spleen the cell membrane of erythrocyte loses competency, and the erythrocyte is destroyed, causing haemolysis. Alternate pathway for the production of NADPH, which is not dependent on G6PD, exists in other human cells but not in erythrocytes, which do not contain mitochondria. For this reason erythrocytes are particularly vulnerable to oxidative destruction. G6PD is therefore important in maintaining cell membrane integrity against the oxidative damage caused by certain drugs or chemicals.⁷

(b) Clinical spectrum of G6PD deficiency related haemolysis

Once it was firmly established that primaquine induces haemolysis in G6PD deficient individuals, it soon became obvious that such individuals were sensitive to other oxidant drugs as well. However, a decade later it was discovered that infection and not necessarily the drug used in treatment was the precipitating factor of haemolysis. We have, therefore, to consider a spectrum of clinical conditions that are associated with G6PD deficiency.

A study was performed in Sabah in Malaysia on 1103 malaria patients, of whom 109 were G6PD deficient, giving a

prevalence of 9.8%. 69 of these G6PD deficient patients were randomly allocated to receive either chloroquine, or chloroquine with primaquine or sulfadoxin-pyrimethamine (Fansidar). In the primaquine group of 23 patients, 16 had complete G6PD deficiency, among whom seven developed haemolysis, severe enough to require blood transfusion in 5, and acute renal failure in 2, one of whom required peritoneal dialysis. No haemolysis occurred in the chloroquine group or the Fansidar group. Primaquine induced haemolysis only occurred in those patients with complete G6PD deficiency and not in those with partial deficiency.⁸

Chan et al described a cross transfusion technique to study the survival of G6PD deficient erythrocytes, labelled with radioactive sodium chromate, and exposed to different drugs. The study was prompted by the fact that most study of drug induced haemolysis in G6PD deficient subjects have been carried out in Negroes with the G6PD A- variant, which is milder and qualitatively different from those in southern Chinese whom they studied. By this technique it was possible to show that in patients with typhoid fever, it was the infection and not the chloramphenicol that shortened the survival of G6PD deficient erythrocytes. While drugs such as primaquine, nitrofurantoin, aspirin and sulphonamides aggravated haemolysis in G6PD deficient Chinese, many drugs do not, including the first line anti-tuberculous drugs, such as streptomycin, isoniazid and PAS, as well as levodopa. In this respect, it is interesting to note that sulphonamide did not cause haemolysis in G6PD deficient malaria patients in Sabah, suggesting there is varying response depending on the type of variants. Chan also pointed out that while chloramphenicol does not shorten the survival of erythrocytes in those with G6PD Canton, G6PD B-Chinese, or G6PD A-, it has been reported to do so in G6PD Mediterranean.¹²

(c) Favism

It has long been known that ingesting fava beans causes haemolytic anaemia in some individuals. While all patients with favism are G6PD deficient, most G6PD deficient individuals can eat fava beans without haemolysis. What additional factor must be present to make G6PD deficient individual develop haemolysis upon ingesting fava beans is still unknown.¹

(d) Neonatal jaundice

Neonatal jaundice associated with G6PD deficiency can be so serious as to cause kernicterus and even death. The



cause of jaundice, however, is not due to haemolysis, because the haemoglobin level and reticulocyte counts of the infants are generally normal. The cause of jaundice is due to the inability of the liver to adequately conjugate bilirubin.¹

Is G6PD Deficiency Protective Against Malaria?

Beutler¹ pointed out the distribution of G6PD deficiency in the world is largely tropical and parallels the distribution of malaria. Since malaria is a disease with high morbidity and high mortality, it therefore has a powerful selective force in human populations. He suggested that the maintenance of a high frequency of G6PD deficiency despite its deleterious effect of haemolysis, is due to its property as a defense against malaria. Luzzatto¹³ distinguished the normal from the G6PD deficient erythrocytes by differential histochemical staining of erythrocytes taken from heterozygous female patients suffering from malaria. They demonstrated that normal erythrocytes were parasitized by malaria parasites to a much greater extent than G6PD deficient erythrocytes. Furthermore G6PD deficient erythrocytes respond to oxidant stress caused by intracellular malaria parasites, with the resultant release of ferriheme. It has been suggested that the excess ferriheme could inhibit the intra-erythrocytic development of malaria parasites in these erythrocytes to account for the reduced parasitaemia in G6PD deficient erythrocytes.¹⁴

Summary

Since the observation of haemolysis caused by Primaquine in 1926, further investigations led to the discovery that the mechanism of haemolysis is due to G6PD deficiency. From that discovery much progress has been made in the precise definition of pathophysiology, and in our knowledge about the gene mutation, the molecular structure of the enzyme, and the population genetics of the enzyme disorder. The link between the global distribution of G6PD deficiency and that of malaria strengthens the clinical importance of the haemolytic potential of primaquine, a widely used anti-malarial drug. Drug induced haemolysis in G6PD deficiency due to G6PD A- is mild and self-limiting, but this is not so in other variants. It is therefore pertinent for each region to study the prevalence of G6PD deficiency and to define the types of G6PD deficiency variants, and their propensity to induce haemolysis in the presence of oxidant drugs. Such knowledge will bring awareness to clinicians to assess the need to screen for G6PD deficiency in patients under their care. Patients screened positive for G6D deficiency should be given a list of oxidant drugs, to avoid.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests