

Cytogenetic, Clinical, Biochemical and Pharmacological Aspects of Glucose 6 Phosphate Dehydrogenase - An Updated Review

Amar Nagesh Kumar^{1*}, Sunil M. Vishwasrao²

¹Department of Biochemistry, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Chinakolambakkam, Madhuranthagam, Tamil Nadu, India

²Department of Pharmacology, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Chinakolambakkam, Madhuranthagam, Tamil Nadu, India

***Corresponding author:** Amar Nagesh Kumar, Department of Biochemistry, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Chinakolambakkam, Madhuranthagam, Tamil Nadu, India. Tel: +91-9944462915; Email: amarnageshkumar@gmail.com

Citation: Kumar AN, Vishwasrao SM (2019) Cytogenetic, Clinical, Biochemical and Pharmacological Aspects of Glucose 6 Phosphate Dehydrogenase - An Updated Review. Adv Biochem Biotechnol 7: ABIO-082. DOI: 10.29011/2574-7258.000082

Received Date: 01 January, 2019; **Accepted Date:** 11 March, 2019; **Published Date:** 19 March, 2019

Abstract

Glucose 6 Phosphate Dehydrogenase (G6PD) is one of the rate-limiting enzymes in hexose monophosphate pathway. G6PD deficiency is an X-linked inheritable abnormality in humans and mostly males are affected, while females are carriers only. The cytogenetic location for all mutations that cause G6PD deficiency is on the band Xq28 of the long arm of the X chromosome, and the locus of the G6PD gene is at Xq27.3 chromosomes. Mutations in the G6PD gene may reduce the amount of G6PD or alter its structure. Most individuals with G6PD deficiency have a qualitative abnormality in the structure of the G6PD enzyme. The deficiency is more commonly found in persons of African, Mediterranean, South Asian, and Middle-Eastern countries. G6PD deficiency is seen to occur most frequently in the areas where malaria is common. G6PD deficiency can cause a spectrum of symptoms that include hemolytic anemia caused by ingestion or exposure to certain triggers. Anemia in turn causes jaundice, pale skin or finger nails, lethargy, fatigue, shortness of breath and fever, among others. The diagnosis of heterozygous deficient women is especially complicated because these women have a normal and a G6PD-deficient population of erythrocytes as a result of lyonization. In this context, knowledge of G6PD enzyme and its deficiency is very essential for clinicians and researchers. Hence an attempt has been made in this article to summarize the cytogenetic, clinical, biochemical and pharmacological aspects of G6PD enzyme.

Keywords: Enzymopathy; Favism; Glucose 6 Phosphate; Lyonization; Malaria; X-linked inheritance

Introduction

Glucose 6 Phosphate Dehydrogenase (G6PD) is one of the rate-limiting enzymes in Hexose Monophosphate Pathway (HMP Pathway) and plays an important role in Red Blood Cells (RBC). Deficiency of G6PD is an X-linked inheritable abnormality in humans. The role of G6PD, advantages of its deficiency has been of interest to researchers and clinicians since its discovery by Dr Ernest Beutler in 1953, and first reported by Alving, et al. in 1956 while investigating the unusual hemolytic reaction that occurred in ethnic black individuals following the administration of primaquine for the treatment of malaria [1,2]. Symptomatic

patients are almost all male persons, due to the X-linked pattern of inheritance. Females are carriers can clinically have affected due to unfavorable lyonization. G6PD deficiency is also known as favism.

Classification

According to the World Health Organization (WHO) G6PD genetic variants classified into five classes [3]. These include:

- Severe deficiency (<10% activity) with chronic (nonspherocytic) hemolytic anemia
- Severe deficiency (<10% activity) with intermittent hemolysis

- Mild deficiency (10-60% activity) and hemolysis seen with stressors only
- Non-deficient variant with no clinical sequel
- Increased enzyme activity with no clinical sequel
- Different gene mutations cause different levels of enzyme deficiency, with classes assigned to various degrees of deficiency and disease manifestation as shown in Table 1 [4,5].

Class	Level of deficiency	Enzyme activity	Prevalence
I	Severe	Chronic nonspherocytic hemolytic anemia in presence of normal erythrocyte function	Uncommon; occurs across populations
II	Severe	Less than 10% of normal	Varies; more common in Asian and Mediterranean populations
III	Moderate	10 to 60% of normal	10% of black males in the United States
IV	Mild to none	60 to 150% of normal	Rare
V	None	Greater than 150% of normal	Rare

Table 1: Degrees of G6PD deficiency.

Cytogenetics and Molecular Aspects of G6PD

More than 140 mutations that cause G6PD deficiency have been identified in the G6PD gene. The cytogenetic location for all mutations that cause G6PD deficiency is on the band Xq28 of the long arm of the X chromosome, and the locus of the G6PD gene is at Xq27.3 chromosomes [4,5]. The molecular location on the X chromosome is base pairs 153,759,605 to 153,775,786. The molecular data on human G6PD shows that for DNA, the size of

gene is 18.5 kilobases and the number of exons and introns are 13 and 12 respectively [4,5]. Size in nucleotides is 2269 for mRNA, and the protein has 515 amino acids [4,5]. G6PD, in its active enzyme form, is made up of either two or four identical subunits, each having a molecular mass of 59,265 kilo-Daltons; this is more than three times that of the hemoglobin molecule [6].

Mutations in the G6PD gene may reduce the amount of G6PD or alter its structure. Most individuals with G6PD deficiency have a qualitative abnormality in the structure of the G6PD enzyme. Many models have been proposed suggesting possible reasons why an abnormal enzyme is not fully active. One of the suggestions is that the decreased stability of a mutated enzyme results either from a change in the conformation of the G6PD molecule or from an increase in its susceptibility to proteolytic enzymes. In either case, the G6PD enzyme is not fully active when it is mutated [6,7]. Wang et al reported the first case of a G6PD-deficient Chinese patient in the category of class I (WHO classification) in the Liaoning Province in northeastern China in 2010 [8,9]. The patient was a 3-year-old boy in whom the G6PD gene had a replacement of G to A at nucleotide 1339. As a result, the amino acid at position 447 should change from Gly to Arg. This replacement is known as G6PD Santiago de Cuba, because it was first discovered in a Cuban boy who showed heavy chronic anemia. Until this report, 28 G6PD variants had been reported in the Chinese population, all of which were in the class II (severe deficiency) or class III (mild deficiency). No case of G6PD deficiency was reported in 414 blood samples from northeastern China. In central China, where falciparum malaria was endemic from the 1950s to 1970s, 2 cases of G6PD deficiency were found amongst 27 blood samples. These cases and the other members from their families had variant type G6PD Kaiping (1388G > T), which is a common variant in the Chinese population [9].

G6PD deficiency is inherited from one or both the parents. It cannot be passed from one person to another in any other way (non-communicable disease). Males can either be G6PD deficient or unaffected. Females can be unaffected or carriers or affected. Probability of inheritance with each pregnancy is summarized in Table 2 [10-13]. The inheritance pattern based on the X-linked recessive nature of the illness is shown in Punnett charts in Tables 3-5.

Father	Mother	Inheritance	
		Son	Daughter
Deficient	Unaffected	Unaffected	carriers
Deficient	Carrier	50% - G6PD deficient	50% - G6PD deficient, 50% - carriers
		50% -unaffected	
Unaffected	Carrier	50% - G6PD deficient, 50% - unaffected	50% - carrier
			50% - unaffected.
Unaffected	G6PD deficient	G6PD deficient	Carriers
Unaffected	Unaffected	All children unaffected	
G6PD deficient	G6PD deficient	All children - G6PD deficient	

Table 2: Inheritance pattern.

		Father	
	Gametes	X_1	Y
Mother	X_1	$X_1 X_1$	$X_1 Y$
	$*X_2$	$X_1 *X_2$	$*X_2 Y$

*Where X_2 is diseased allele; X_1 is normal X allele. Green color shade represents Female offspring; Red Color shade represents Male offspring. 50% daughters are carriers and 50% daughters are normal. Similarly 50% of the sons are normal and 50% are affected.

Table 3: Punnet chart showing the different possible offspring if the mother is having diseased X-allele.

		Father	
	Gametes	$*X_2$	Y
Mother	X_1	$X_1 X_2$	$X_1 Y$
	X_1	$X_1 X_2$	$X_1 Y$

*Where X_2 is diseased allele; X_1 is normal X allele. Green color shade represents Female offspring; Red Color shade represents Male offspring. Daughters are 100% carriers whereas sons are absolutely normal.

Table 4: Punnet chart showing the different possible offspring if the father is having diseased X-allele.

		Father	
	Gametes	$*X_2$	Y
Mother	X_1	$X_2 X_1$	$X_1 Y$
	$*X_2$	$X_2 X_2$	$X_2 Y$

*Where X_2 is diseased allele; X_1 is normal X allele. Green color shade represents Female offspring; Red Color shade represents Male offspring. 50% daughters are carriers and 50% daughters are affected. Similarly, 50% of the sons are normal and 50% are affected.

Table 5: Punnet chart showing the different possible offspring if both the parents are having diseased X-allele.

Clinical Aspects of G6PD

Prevalence

Glucose 6-phosphate dehydrogenase is the most common human enzyme defect being present in more than 400 million people worldwide [14,6]. The deficiency is more commonly found in persons of African, Mediterranean, South Asian, and Middle-Eastern countries [15]. G6PD deficiency is also prevalent in Sardinia, Italy. WHO data from 1989 reported the prevalence of 0.39% in Europe, which represents in France 120,000 deficient male patients and approximately 1,400 new cases among male newborn babies. From technologically deficient areas in the world, the reporting of the G6PD deficiency may be inadequate. While Philippines had few or no cases of G6PD deficiency earlier, now that newborn screening has been implemented, 12% of its population is reported to be affected. A report in 2000 mentioned an estimated 225,000 males and 92,000 females were affected in Greece [16]. Reportedly, 1 in 10 African-American males in United States is affected by this condition. It may be seen in about 20% population in Africa, 4-30% in Mediterranean, and in Southeast Asians. A study from Solomon Islands in 8541 individuals (3880 males and 4636 females), found 20.3% overall prevalence (21.7% in males and 19.1% in females) of G6PD deficiency and 6.9% prevalence of severe deficiency (less than 10% of normal enzyme activity) [17]. Cases of sporadic gene mutation may occur in all populations.

The two most common variants of G6PD deficiency are G6PD Mediterranean and G6PD-A variant. G6PD Mediterranean variant falls in WHO Class II and mainly affects Italian, Grecian, Spanish, and the individuals from middle eastern descent such as Arabic, Kurdish or Sephardic Jewish populations. In this variant, the neonatal hyperbilirubinemia is known to occur and may be more severe, and favism is more common [18-21]. The G6PD-A variant falls in WHO Class III and mainly affects the population of African descent. Neonatal hyperbilirubinemia may be seen in this variant also, and favism is less common. Hemolysis on exposure to oxidative stress is seen in both these variants [17,22-24].

Clinical Features of G6PD Deficiency

So far more than 440 different variants of enzyme G6PD have been identified with level of deficiency ranging from severe to mild. Of which around 80 G6PD variants are associated with chronic non-spherocytic hemolytic anemia [7,15,18]. Most individuals with G6PD deficiency are asymptomatic (can be homozygous or heterozygous) [18-20]. G6PD deficiency can cause a spectrum of symptoms that include hemolytic anemia caused by ingestion or exposure to certain triggers. Anemia in turn causes jaundice, pale skin or finger nails, lethargy, fatigue, shortness of breath and fever, among others. These symptoms usually go away on their own when exposure to the trigger is removed [16,19-20].

In newborns, G6PD deficiency may cause persistent jaundice. If left untreated, this may lead to brain damage (kernicterus) and mental retardation [8]. Individuals with the disease may exhibit non-immune hemolytic anemia in response to a number of causes, most commonly exposure to certain medications (antimalarial or antihistamine/anti pyritic drugs or chemicals or bacterial or viral infections [21-24].

Biochemistry of G6PD

The enzyme Glucose-6-Phosphate Dehydrogenase (G6PD) is a dimer and tetramer, taking part in the step in the Hexose-Monophosphate (HMP) pathway. G6PD has 400 isoforms [8,16-17]. Among all, the G6PD in RBC is 351 ± 60.6 U/ 10^{12} RBC [8,14]. It is an NADP dependent enzyme and is involved in maintenance of RBC membrane integrity. Due to the presence of oxygen, hydrogen peroxide is continuously formed inside the RBCs. Hydrogen peroxide destroys bio membranes and therefore there is lysis of RBCs. This is prevented by a process involving glutathione and NADPH. The biochemical reaction involving G6PD is shown in Figure 1. In the G6PD deficient individuals, the normal in vivo life half-life of RBCs of 62 days' decreases to as less as 13 days, resulting in mild hemolysis and is aggravated by exposure to certain drugs such as primaquine [10].

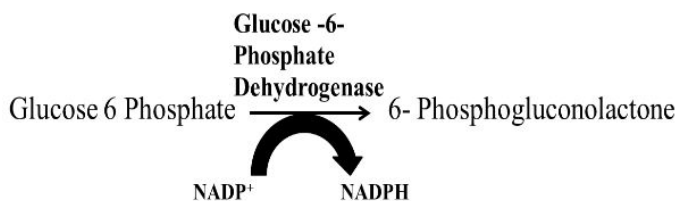


Figure 1: The biochemical reaction catalyzed by the enzyme G6PD.

Role of G6PD in Various Functions

Embryo Protective Role of G6PD

Glucose-6-phosphate dehydrogenase is known to be cytoprotective against oxidative stress especially to the red blood cells. Nicol et al showed that litters from untreated pregnant mutant mice with a hereditary G6PD deficiency had increased prenatal (fetal resorptions) and postnatal death. When treated with the anticonvulsant drug phenytoin, G6PD-deficient dams had higher embryonic DNA oxidation and more fetal death and birth defects. The reported G6PD gene mutation was confirmed and used to genotype fetal resorptions, which were primarily G6PD deficient. This study was claimed to be the first evidence that G6PD is a developmentally critical cytoprotective enzyme for both endogenous and xenobiotic-initiated embryo pathic oxidative stress and DNA damage, and suggested that G6PD deficiencies

accordingly may have a broader biological relevance as important determinant of infertility, in utero and postnatal death, and teratogenesis [18].

G6PD Deficiency as an Advantage

Deficiency of glucose-6-phosphate dehydrogenase enzyme is thought to pose advantages in certain situations. Suggested situations include malaria, retinal vein occlusion, and ischemic heart and cerebrovascular disease [19].

G6PD deficiency and retinal vein occlusion. A study from Sardinia (Italy) showed that the patients deficient in G6PD were found to have a significantly lower risk of developing Retinal Vein Occlusion (RVO) [19].

G6PD Deficiency and Malaria

G6PD deficiency is seen to occur most frequently in the areas where malaria is common. The researchers believe that the carriers of a G6PD mutation may be partially protected against malaria. The fact that the prevalence of G6PD deficiency correlates with endemicity for malaria had led to the hypotheses that G6PD deficiency may be result of natural selection conferring protection against malaria infection [20-23]. However, studies have indicated that this protection may only occurs with certain G6PD variants, and that differing level of protection are likely to be seen in hemizygous males, homozygous and heterozygous females [24].

The incidence of the most common form of glucose 6-phosphate dehydrogenase deficiency, characterized by a tenfold reduction in enzymatic activity in red blood cells, is 11% among Americans of African heritage. This high frequency suggests that the deficiency may be advantageous under certain environmental conditions. Indeed, glucose 6-phosphate dehydrogenase deficiency protects against falciparum malaria [25-27]. The parasites causing this disease to require reduced glutathione and the products of the pentose phosphate pathway for optimal growth. Thus, glucose 6-phosphate dehydrogenase deficiency is a mechanism of protection against malaria, which accounts for its high frequency in malaria-infested regions of the world [28]. So also, a reduction in the amount of functional G6PD appears to make it more difficult for the malarial parasite to invade red blood cells. Another theory says that the cells infected with the *Plasmodium* parasite are cleared more rapidly by the spleen. This phenomenon might give G6PDH deficiency carriers an evolutionary advantage by increasing their fitness in malarial endemic environments. Because hemolysis affects mature red blood cells more readily, there are fewer of them to host malaria parasites. When an infected RBC dies before the parasite is ready, the malaria parasite dies as well and it does not have the chance to produce the poisons. Because of this the typical symptoms do not usually manifest themselves in G6PD deficient patients. Atypical symptoms could make malaria more difficult to diagnose and account for the belief that it is less

prevalent in G6PD Deficient patients. Malaria can still sequester in the liver however. The dangerous part is that a person can die or become very ill from hemolysis and G6PD deficiency patients cannot take antimalarial. All of the antimalarial listed by the CDC are contraindicated except Doxycycline, which cannot be taken by pregnant women or children under the age of eight. People must limit their sun exposure while on the drug as well [4,14,26].

Hemolysis in G6PD Deficiency Can Manifest in Many Ways

Acute hemolysis is caused by exposure to an oxidative stress or in the form of an infection, oxidative drugs or fava beans. Acute hemolysis, which is self-limiting, may be rarely severe enough to warrant blood transfusion. Neonatal hyperbilirubinemia may require phototherapy or exchange transfusion to prevent kernicterus. The variant causing chronic hemolysis is uncommon because it is related to sporadic /9gene mutation rather than the more common inherited gene mutation [15].

They include

- Prolonged neonatal jaundice in turn may lead to kernicterus, the most serious complication of G6PD deficiency.
- Hemolytic anemia in response to
- Unusual complication of infections
- Certain drugs
- Certain foods (favabeans)
- Chemicals
- Diabetic ketoacidosis

Important Drugs Cause Hemolysis Due to G6D Deficiency

Many drugs are potentially harmful to people with G6PD deficiency, and they produce oxidative damage to erythrocytes leading erythrocyte destruction [22-24]. Hemolysis is typically known to occur 24-72 hours after ingestion and resolves within 5-7 days [23]. Oxidative drugs are known to cross the milk barrier and produce oxidative damage and hemolysis in the breast-fed infant [25]. Some of the drugs which can cause hemolysis in G6PD deficient subjects are listed below.

- **Antimalarials:** Primaquin, Pamaquin and chloroquin
- **Analgesics:** Acetylsalicylic acid, Phenacetin, Phenazopyridine, Acetanilide
- **Sulphonamides:** Sulphanilamide, Sulphamethazole and Mafenide
- **Sulphones:** Thiazole sulphone, Dapsone, Sulphoxone
- **Nitrofurans:** Furadantin, Furoxone [23-25,27,28]

Fava beans ingestion has been identified to precipitate the symptoms of G6PD deficiency in some G6PD deficient individuals, although all individuals may not exhibit hemolysis upon fava beans ingestion [1,4,11,20]. Favism is most common in individuals with G6PD class II variants, but rarely occurs in individuals with G6PD A-variant. Although the exact ingredient in fava beans responsible for oxidative damage is unknown, it could be possibly vicine, convicine, or isouramil. Fava beans are also known as Bell beans or Horse beans. Bell beans are known by various names that include broad beans, English dwarf beans, fever beans or haba beans. Horse beans are known by different names including pigeon beans, silk worm beans or tick beans [29-31].

Diagnosics - Testing Methods

The diagnosis of heterozygous deficient women is especially complicated because these women have a normal and a G6PD-deficient population of erythrocytes as a result of lyonization. Hence some researchers concluded that two different tests for diagnosing men and women is the ideal approach. The fluorescent spot test has the advantage of being less expensive and easy to perform, but is only reliable for discriminating hemizygous G6PD-deficient men from non-deficient men. Cytochemical assay is supposed to be the only reliable assay to discriminate between heterozygous-deficient women and non-deficient women or homozygous-deficient women. Hence the cytochemical assay is recommended for women, although it is more expensive and difficult to perform, and a need was expressed to make it more simplified especially keeping in mind the affordability issues in the developing countries [32].

Direct testing of the enzymatic activity of G6PD on a freshly collected blood sample is the most widely used diagnostic method for diagnosis of deficiency. Methods used include older tests such as brilliant cresyl blue de-colorization test and methaemoglobin reduction test [16,17]. The methodology recommended by the International Committee for Standardization in Hematology is the NADPH fluorescent spot test, which requires a UV lamp [18,19]. Suboptimal assay performance due to very wet weather conditions and bloodspot sample collection on rainy or humid days is known. Drying is an important step, especially for the storage and consistent elution for the assay [30]. Both of these considerations may have confounded measurement of G6PD activity, and have led to a greater proportion of individuals showing lower normal activity or moderate deficiency. To minimize these issues, it is recommended that filter paper be stored in zip-lock bags containing silica gel desiccants before being used for blood collection, and perhaps change of silica gel desiccants frequently after collection for samples that were not able to be dried sufficiently due to wet weather conditions [30,31].

These methods all have shortcomings that limit their use in mass-screening or in field settings [18]. Other methods that

do not require UV lamp have been used for screening studies include the ring method and Sephadex gel MTT-PMS method [33-36]. These methods are also used as a diagnostic test prior to primaquine treatment in larger hospitals and health centers in developing countries, where the necessary facilities and equipment are available. Other methods that have been described for testing include cytochemical assays [25], and DNA sequence analysis of the G6PD gene. The former has the advantage of being a reliable method for detection of hemizygous deficient males, homozygous deficient females, or heterozygous deficient females because the G6PD status of individual erythrocytes is tested [26]. DNA sequence analysis requires analysis of the whole gene which spans 18kB of genomic sequence [1,22-25]. However, all of these tests suffer from limitations that inhibit their utility for in-field mass-screening purposes, due to factors such as technical expertise required, expense, duration of test procedure, sensitivity of reagents to light and heat, low detection threshold, or relatively low throughput capacity [22,24].

Kuwahata, et al. described a new method modified from the WST8 method optimized to a 96-well plate format, using dried blood spots with internal standards as controls. This new method was evaluated to determine the prevalence of G6PD deficiency in Isabel Province, Solomon Islands [17].

Tests to be Carried Out, To Confirm the Deficiency of G6PD Enzyme Will Include

There are number of screening tests to detect G6PD deficiency. Conversion of Nicotinamide Adenine Dinucleotide Phosphate (NADP) to its reduced form (NADPH) in erythrocytes is the basis of diagnostic testing for the deficiency. A simple direct test for G6PD is the fluorescent spot test which has largely replaced the older tests. This test was developed by Beutler and Mitchell which is based on the fluorescence of NADPH, generated by an adequate amount of G6PD enzyme [34].

Principle of the Test

The principle of the Ani Lab Systems Neonatal G6PD assay is based on an enzymatic method intended for the quantitative determination of glucose 6-phosphate dehydrogenase activity from dried blood spots. NADP⁺ is reduced by G6PD (glucose 6-phosphate dehydrogenase) in the presence of G6-P (Glucose 6-Phosphate), and the rate of formation of NADPH is proportional to the G6PD activity, and is determined fluorometrically (see below). Cold copper reagent is added to stop the reaction and stabilize the fluorescent complex. Fluorescence is measured (λ_{ex} 355 nm, λ_{em} 460 nm).

- The other tests are direct DNA testing and or sequencing of the G6PD gene.
- Along with these test other tests to confirm the deficiency of G6PD include complete blood count and reticulocyte count [27,31]

This test measures the amount of Glucose-6-Phosphate Dehydrogenase (G6PD) in the Red Blood Cells (RBCs). G6PD is an enzyme that protects red blood cells from the effects of oxidation [29,31,33]. If there is insufficient G6PD, the RBCs become more vulnerable to oxidative damage. If these RBCs are exposed to an oxidative agent (see a list of drugs and foodstuff to avoid), it changes their cellular structure, precipitating hemoglobin inside the cells (Heinz Bodies), causing them to break apart [35,37].

Neonatal Screening Issues

Neonatal screening, for this disease, has long been established in many countries and the method most commonly used is the semi-quantitative method described by Beutler [34] or modifications to this method. This method was believed to discriminate between deficient (partial or total deficiency) and normal cases.

Prognosis

Spontaneous recovery from hemolytic episode is known in the prognosis of this condition. Renal failure or death following a severe hemolytic event is known as a rare complication. G6PD deficient individuals do not appear to acquire any illness more frequently than other people and may have less risk than other people for acquiring ischemic heart disease and cerebrovascular disease.

Treatment

Prevention is better than cure

- Avoidance of the drugs and foods that cause hemolysis
- Vaccination against some common pathogens may prevent infection induced attacks.
- In the acute phase of hemolysis, patients may benefit from removal of the spleen as this is an important site of red cell destruction.
- In case of a severe form, a blood transfusion, or even an exchange transfusion, may be required.
- Folic acid should be used in any disorder featuring a high red cell turn over.

Conclusion

Newborns and infants should be screened for G6PD deficiency when family history, ethnic or geographic origin, or the timing of appearance of neonatal jaundice suggests possibility of G6PD deficiency. G6PD deficiency can be diagnosed with a quantitative spectrophotometric analysis or more commonly by a rapid fluorescent spot test. The patients with G6PD deficiency should avoid exposure to oxidative drugs and ingestion of fava beans.

Acknowledgements

We are thankful to our Managing Director Dr Regupathy Annamalai, M. S, M. Ch for providing us the internet facility and allowing us to access paid journals contents in the library, to accomplish this article.

Financial Support: We did not receive any external funding.

References

1. Beutler E (2008) Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood* 111: 16-24.
2. Carson PE, Flanagan CL, Ickes CE, Alving AS (1956) Enzymatic deficiency in primaquine sensitive erythrocytes. *Science* 124: 484-485.
3. Mazza, Joseph (2002) *Manual of Clinical Hematology*. Lippincott Williams & Wilkins: 101-102.
4. Ruwende C, Hill A (1998) Glucose-6-phosphate dehydrogenase deficiency and malaria. *J Mol Med* 76: 581-588.
5. Gregg XT, Prchal JT (2000) Red cell enzymopathies. In: Hoffman R, ed. *Hematology: Basic Principles and Practice*. 4th ed. Philadelphia. Churchill Livingstone: 657-660.
6. Scriver C, Beaudet A, Sly WS, Valle D (Eds) (1995) Glucose 6-phosphate dehydrogenase deficiency" In: *The metabolic and molecular bases of inherited disease*. 7th ed n.p.: McGraw-Hill, Inc. New York: 3367-98.
7. Beutler E (1994) G6PD deficiency. *Blood* 84: 3613-3636.
8. Gregg XT, Prchal JT (2000) Red cell enzymopathies. In: Hoffman R, ed. *Hematology: Basic Principles and Practice*. 4th ed. Philadelphia. Churchill Livingstone: 657-660.
9. Wang J, Matsuoka H, Hirai M, Mu L, Yang L, et al. (2010) The first case of a class I glucose-6-phosphate dehydrogenase deficiency, G6PD Santiago de Cuba (1339 G > A), in a Chinese population, as found in a survey for G6PD deficiency in northeastern and central China. *Acta Med Okayama* 64: 49-54.
10. Yoshida A, Stamatoyannopoulos G, Motulsky AG (1967) Negro variant of glucose 6-phosphate dehydrogenase deficiency (A-) in man. *Science* 155: 97-99.
11. Nantakomol D, Paul R, Palasuwan A, Day NP, White NJ, et al. (2013) Evaluation of the phenotypic test and genetic analysis in the detection of glucose-6-phosphate dehydrogenase deficiency. *Malar J* 12: 289
12. Sabeti PC, Schaffner SF, Fry B, Lohmueller J, Vavilily P, et al. (2006) Positive natural selection in the human lineage. *Science* 312: 1614-1620.
13. Sukumar S, Mukherjee MB, Colah R, Mohanty D (2004) Molecular basis of G6PD deficiency in India. *Blood Cells Mol Dis* 33: 141-145.
14. Cappellini MD, Fiorelli G (2008) Glucose 6-phosphate dehydrogenase deficiency. *Lancet* 371: 64-74.
15. Frank JE (2005) Diagnosis and management of G6PD deficiency. *American Family Physician* 72: 1277-1282.

16. Belfield KD, Tichy EM (2018) Review and drug therapy implications of glucose-6-phosphate dehydrogenase deficiency. *Am J Health Syst Pharm* 75: 97-104.
17. Kuwahata M, Wijesinghe R, Ho MF, Pelecanos A, Bobogare A, et al. (2010) Population screening for glucose-6-phosphate dehydrogenase deficiencies in Isabel Province, Solomon Islands, using a modified enzyme assay on filter paper dried bloodspots. *Malaria Journal* 9: 223.
18. Nicol CJ, Zielenski J, Tsui L, Wells PG (2000) An embryoprotective role of glucose-6-phosphate dehydrogenase in developmental oxidative stress and chemical teratogenesis. *The FASEB journal* 14: 111-127.
19. Pinna A, Carru C, Solinas G, Zinellu A, Carta F, et al. (2007) Glucose-6-phosphate dehydrogenase deficiency in retinal vein occlusion. *Investigative ophthalmology and visual science* 48 : 2747-2752.
20. WHO Working Group (1989) Glucose-6-phosphate dehydrogenase deficiency. *Bull World Health Organ* 67: 601-611.
21. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E, et al. (2009) The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells Mol Dis* 42: 267-278.
22. Denic S, Nicholls MG (2007) Genetic benefits of consanguinity through selection of genotypes protective against malaria. *Hum Biol* 79: 145-158.
23. Ganczakowski M, Town M, Bowden DK, Vulliamy TJ, Kaneko A, et al. (1995) Multiple glucose 6-phosphate dehydrogenase-deficient variants correlate with malaria endemicity in the Vanuatu archipelago (southwestern Pacific). *Am J Hum Genet* 56: 294-301.
24. Guindo A, Fairhurst RM, Doumbo OK, Wellems TE, Diallo DA, et al. (2007) X-linked G6PD deficiency protects hemizygous males but not heterozygous females against severe malaria. *PLoS Med* 4: e66.
25. Tripathy V, Reddy BM (2007) Present status of understanding on the G6PD deficiency and natural selection. *J Postgrad Med* 53: 193-202.
26. Chu CS, Bancone G, Moore KA, Win HH, Thitipanawan N, et al. (2017) Haemolysis in G6PD Heterozygous Females Treated with Primaquine for *Plasmodium vivax* Malaria: A Nested Cohort in a Trial of Radical Curative Regimens. *PLoS Med* 14: e1002224.
27. Howes RE, Battle KE, Satyagraha AW, Baird JK, Hay SI, et al. (2013) G6PD deficiency: Global distribution, genetic variants and primaquine therapy. *Adv Parasitol* 81: 133-201.
28. Berg JM, Tymoczko JL, Stryer L (2002) *Biochemistry*. 5th edition. New York: W H Freeman.
29. Mason PJ (1996) New insights in to G6PD deficiency. *Br J Hematol* 94: 585-591.
30. Youngster I, Arcavi L, Schechmaster R (2010) Medications and glucose-6-phosphate dehydrogenase deficiency: an evidence based review. *Drug Saf* 33: 713-726.
31. Luzzatto L, Seneca E (2014) G6PD deficiency: A classic example of pharmacogenetics with on-going clinical implications. *Br J Haematol* 164: 469-480.
32. Piomelli S, Corash LM, Davenport DD, Miraglia J, Amorosi EL, et al. (1968) In vivo lability of glucose-6-phosphate dehydrogenase in GdA- and Gdmediterranean deficiency. *J Clin Invest* 47: 940-948.
33. Peters AL, Van Noorden CJF (2009) Glucose-6-phosphate dehydrogenase deficiency and malaria: Cytochemical detection of heterozygous G6PD deficiency in women. *J Histochem Cytochem* 57: 1003-1011.
34. Beutler E, Mitchell M (1968) Special modifications of the fluosercent screening method for glucose 6-phosphate dehydrogenase deficiency. *Blood* 32: 816-818.
35. Reclos GJ, Hatzidakis CJ, Schulpis KH (2000) Glucose-6-phosphate dehydrogenase neonatal screening: Preliminary evidence that a high percentage of partially deficient female neonates is missed during routine screening. *Journal of Medical Screening*. 7: 46-51.
36. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia (2004) Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics* 114: 297-316.
37. Bancone G, Chu CS, Chowwiwat N, Somsakchaicharoen R, Wilaisrisak P, et al. (2015) Suitability of Capillary Blood for Quantitative Assessment of G6PD Activity and Performances of G6PD Point of Care Tests. *Am J Trop Med Hyg* 92: 818-824.