REVIEW ARTICLE

HAEMOLYSIS WITH ANTI-MALARIAL DRUGS IN GLUCOSE 6 PHOSPHATE DEHYDROGENASE DEFICIENCY

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Introduction

Development of haemolysis in any febrile patient is a cause of concern for a physician. However with careful history and examination the underlying cause can be detected. The wide spread empirical use of anti-malarial drugs and NSAIDs in febrile patients can result in haemolysis in susceptible population.

G6PD deficiency leads to haemolysis in patients whenever they are exposed to oxidative stress in the form of drugs. There is a long list of drugs, which can cause this dreadful complication and serious problems for the patients. These drugs are to be avoided in G6PD deficient patients but it is difficult to anticipate this complication. Since it is difficult to predict which patient might develop this complication when drugs like NSAIDs and anti-malarial drugs are used in febrile patients, it is important to be careful while using these drugs in susceptible population.

Key words: G6PD, anti-malarial drugs, haemolysis

Epidemiology

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is the commonest defect on Hexos Monophosphate Shunt resulting in oxidative damage to RBC membrane and resultant haemolysis. It is an X-linked disorder, is the most common enzymatic disorder of red blood cells in humans, affecting 200 to 400 million people.[1,2]

There are around 30 different variant of the enzyme G6PD. The normal G6PD is type B whereas G6PD A+ is the most common variant found in 20% blacks and is functionally normal. A – variant is seen in 11% American blacks. Other variants are common in Mediterranean and Chinese population. Normally G6PD activity decreases to 50% during the 120 days life span of normal RBC. The problem arises when these patients are exposed to environmental stress.[3]

Function of Glucose 6 Phosphate Dehydrogenase enzyme

Glucose-6-phosphate dehydrogenase catalyzes the initial step in the hexose monophosphate (HMP) shunt, oxidizing glucose-6-phosphate to 6- phosphogluconolactone and reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. The main function of the HMP shunt is to protect red blood cells against oxidative injury via the production of NADPH. Red blood cells contain relatively high concentrations of reduced glutathione (GSH) which is protective against oxidant injury. The oxidants, such as super oxide anion (O2-) and hydrogen peroxide, are formed within red cells due to reactions of hemoglobin with oxygen and due to drugs or infections. They accumulate within red cells and cause oxidation of hemoglobin and other proteins leading to loss of function and cell death.

Under normal circumstances, these compounds (oxidants) are rapidly inactivated by GSH in conjunction with glutathione peroxidase. These reactions result in the conversion of GSH to oxidized

glutathione (GSSG). The depleted GSH levels are restored by glutathione reductase which catalyzes the reduction of GSSG to GSH. This reaction requires the NADPH generated by G6PD.

The combined effect of HMP shunt to glutathione metabolism is responsible for protecting intracellular proteins from oxidative stress. G6PD deficiency results in hemolytic episodes related to altered HMP shunt and glutathione metabolism. The cells that overexpress G6PD are protected against oxidative injury.[4] The gene for G6PD has been identified and is located on the X chromosome (band X q28).[5]

Classification

The World Health Organization has classified the different G6PD variants according to the magnitude of the enzyme deficiency and the severity of hemolysis. Classes IV and V are of no clinical significance.[6,7]

- Class I: which are rare, have severe enzyme deficiency (less than 10 percent of normal) and have chronic hemolytic anemia
- Class II: severe enzyme deficiency, but there is usually only intermittent hemolysis
- Class III: moderate enzyme deficiency (10 to 60 percent of normal) with intermittent hemolysis usually associated with infection or drugs
- Class IV: no enzyme deficiency or hemolysis
- Class V: increased enzyme activity.

Hemolysis in G6PD deficiency

G6PD deficiency is expressed in males carrying a variant gene that produces sufficient enzyme deficiency to lead to symptoms. In comparison, heterozygous females are usually clinically normal. However, the mean red blood cell enzyme activity in heterozygous females may be normal, moderately reduced, or grossly deficient depending upon the degree of lyonization and the degree to which the abnormal G6PD variant is expressed.

The process of haemolysis can be triggered by a variety of insults including exposure to drugs, toxins or viral/ bacterial infections. The interaction of oxygen with heme in presence of these offending agents, results in production of oxidants. These oxidants are not effectively neutralized due to deficiency of G6PD, resulting in cellular damage and death of RBCs. Certain drugs (Table 1) and toxins, having high redox potential, like primaquine and naphthalene may cause severe haemolysis.

The most commonly affected organ due to intravenous haemolysis is Kidney. This leads to toxic damage to renal tubules and resultant acute renal failure.[3] Metabolic acidosis, secondary to acute renal failure or sepsis due to infections, may also contribute towards haemolysis in G6PD deficient patients.

The disease is self limiting and total RBC mass decreases by only 25 to 30 %. The diagnosis of G6PD deficiency may be missed during the acute hemolytic episode due to the young RBCs having good enzyme activity. However this might be detected at a later stage when patient is asymptomatic. This calls for a detailed investigation of the patient with acute attack of haemolysis at a later stage.[3,8]

Clinical presentation

The course of an acute hemolytic episode following the administration of offending drugs to subjects with G6PD deficiency is variable. The classical hemolytic episode has been described after exposure to Primaquine.

There is the sudden onset of jaundice, pallor, and dark urine, with or without abdominal and back pain usually two to four days after drug ingestion,. This is associated with an abrupt fall in the hemoglobin concentration and the peripheral blood smear reveals cell fragments, microspherocytes, and eccentrocytes. The damaged red cells get sequestrated in both the liver and spleen.[9]

Stimulation of erythropoiesis due to haemolysis and resultatnt anemia reflects as, increase in reticulocytes that is apparent within five days and is maximal at seven to ten days after the onset of hemolysis.

The reason for self-limiting episode could be mild deficiency of G6PD, which resulted in haemolysis of only older RBCs[10] or prompt withdrawal of offending drug. Even with continued drug exposure, the acute hemolytic process ends after about one week with reversal of the anemia. This is due to the fact that the younger crops of RBCs have good activity of enzyme and are not much prone to haemolysis.[5] The enzymatic activity of G6PD is normal in reticulocytes, but declines rapidly thereafter, with a half-life of 13 days (normal about 62 days).[12,13]

Diagnosis of G6PD deficiency

The diagnosis of G6PD deficiency is made by adding a measured amount of hemolysate to an assay mixture that contains substrate (glucose-6-phosphate) and cofactor (NADP); the rate of NADPH generation is measured spectrophotometrically.[14] A number of other screening tests that are also available[7] but the fluorescent spot test is the simplest, most reliable, and most sensitive of the G6PD screening tests.[15] It is based upon the fluorescence of NADPH after glucose-6-phosphate and NADP are added to a hemolysate of test cells.

Other screening tests estimate NADPH generation indirectly by measuring the transfer of hydrogen ions from NADPH to an acceptor. In the methemoglobin reduction test, methylene blue is used to transfer hydrogen from NADPH to methemoglobin, thereby promoting its reduction.[7,16] When combined with a technique for the elution of methemoglobin from intact cells, this test can detect relative G6PD sufficiency in individual red cells; this permits detection of the carrier state with approximately 75 percent accuracy.[17]

Screening for G6PD deficiency

Routine screening for G6PD deficiency is neither performed nor advocated. The molecular techniques for prenatal diagnosis have not been developed because of the fact that most common variants that cause acquired hemolytic anemia pose little health hazard. The class I G6PD variants associated with chronic haemolysis are so rare that screening for it is impractical. However, certain populations, in whom the prevalence of G6PD deficiency is high, might benefit from detection of G6PD deficiency so they can avoid obvious oxidant exposures.

G6PD deficiency and anti-malarial drugs

Acute hemolytic episodes are very well described with antimalarial drugs and Primaquine is the commonest drug implicated.[18,19] Majority of the cases in literature incriminate primaquine for haemolysis in patients with malaria.[20]

However, primaquine, has been safely given to individuals with the G6PD A- variant as long as a low dose is used (15 mg/day or 45 mg once or twice weekly) under close supervision of blood counts.[21] The mild anemia that may result is corrected by the compensatory increase in erythropoietin secretion and does not recur unless the dose of drug is escalated. Transfusions are required only if there is massive haemolysis or erythropoiesis is impaired.

The literature incriminating chloroquine for haemolysis is scarce, few reports from India and Afganistan have shown chloroquine administration in G6PD deficient children resulting in intravascular haemolysis and acute renal failure.[22,23]

In the study from Afganistan 11 out of 28 children developed intravenous haemolysis after antimalarial drugs. 9 patients received chloroquine alone, and one each was given chloroquine in combination with other drugs like aspirin and chloramphenicol.[22]

In a study from Sabah 109 (9.8%) of 1103 malaria patients were deficient in glucose-6-phosphate dehydrogenase (G6PD). 69 of these G6PD-deficient patients were randomly allocated to 1 of 3 treatment regimes with chloroquine, chloroquine and primaquine, or sulfadoxine-pyrimethamine (Fansidar). No hemolysis was observed in the chloroquine group. Except for a single mild case, no case of hemolysis was seen in the group getting chloroquine and fancidar. However, in the 2nd group of 23 patients, hemolysis occurred in 7 of 16 patients who had complete G6PD deficiency. Of these 7, 5 required blood transfusion and the other 2 developed acute renal failure requiring peritoneal dialysis.[19]

Some experts still believe that chloroquine & quinine both can be safely given in patients with G6PD deficiency.[8] Even though haemolysis is uncommon with chloroquine, the most appropriate approach would be to watch the patients receiving it closely for evidence of intravenous haemolysis.[18] Though there are reports of black water fever when severe facliparum infection is treated with mefloquine, resulting in severe haemolysis but its association with G6PD deficiency in these patients is speculative.[24,25]

All the other anti-malarial drugs like halofantrine, artemisenine derivatives, proguanil, atovoquon are considered safe and there is no report of haemolysis with these drugs when used in G6PD deficient patients.

Prevention of hemolysis in susceptible patients

Since prevention is the best policy the G6PD deficient subjects should be warned regarding the use of certain drugs and should be given a list of safe and unsafe drugs so as to prevent these attacks in future. In addition, pregnant and nursing women, who are heterozygous for G6PD deficiency should avoid drugs with oxidant potential, because some of these drugs gain access to the fetal circulation and to breast milk.

When hemolytic process occurs in patients with G6PD deficiency the inciting agent drug/infection is to be removed. However in class 3 variant such as G6PD A- type the essential drug can be continued with a strict watch kept on blood counts and hemoglobin.

Conclusion

The aim of this review is to make physicians aware of the fact that G6PD deficiency is difficult to anticipate. Development of haemolysis in a febrile patient must alert the physicians regarding the presence of this deficiency and every effort must be directed at finding the underlying cause so as to prevent future complications.

Haemolysis due to G6PD deficiency must be differentiated from IV haemolysis due to complicated malaria in patients coming from endemic areas.

There is enough evidence suggesting that primaquine causes haemolysis but evidence in favor of chloroquine ingestion resulting in haemolysis in G6PD deficient patients is scanty. It may still be wiser to use quinine or alternative agents, which are safe in such patients or observe patients carefully when ever chloroquine is used empirically.

Common drugs causing hemolysis in G6PD deficient patients[3,8]

1. Antimalarials: Primaquine, Pamaquine
2. Sulfa drugs: Sulfonamide, Sulfamethoxazole
3. Dapsone
4. Nitrofurantoin
5. Analgesics: Acetanilide
6. Vitamin K
7. Doxorubicin
8. Methylene blue
9. Nalidixic acid
10. Furazolidine, Niridazole
11. Phenazopyridine (Pyridium)
12. Isobutyl nitrate
13. Sulfapyridine
14. Thiazosulfone
15. Phenylhydrazine
16. Toluidine blue

Drugs that can be safely given in therapeutic dosage in patients of G6PD deficiency with nonspherocytic hemolytic anemia[8]

1. Acetaminophen
2. Acetophenetidine (Phenacetin)
3. Aminopyrine
4. Actazoline
5. Antipyrine
6. Ascorbic acid (vitamin C)
7. Benzhexol
8. Chloramphenicol
9. Chlorguanidine (Proguanil)
10. Colchicin
11. Diphenylhydramine
12. Isoniazide
13. Levo dopa
14. Menapathine
15. P-Aminobenzoic acid

16. Phenylbutazone
17. Phenytoin
18. Probenacid
19. Procainamide
20. Pyrimethamine
21. Quinine, Qunidine, Chloroquine, Mefloquine
22. Proguanil
23. Halofantrine
24. Streptomycin
25. Sulfacytine
26. Sulfadiazine, Sulfamerazine, Sulfisoxazole
27. Trimethoprim
28. Vitamin K

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