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Glucose-6-Phosphate Dehydrogenase-Deficiency in Transfusion Medicine: The Unknown Risks

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Abstract

The hallmark of glucose-6-phosphate dehydrogenase (G6PD) deficiency is red blood cell (RBC) destruction in response to oxidative stress. Patients requiring RBC transfusions may simultaneously receive oxidative medications or have concurrent infections, both of which can induce hemolysis in G6PD-deficient RBCs. Although it is not routine practice to screen healthy blood donors for G6PD deficiency, case reports identified transfusion of G6PD-deficient RBCs as causing hemolysis and other adverse events. In addition, some patient populations may be more at risk for complications associated with transfusions of G6PD-deficient RBCs because they receive RBCs from donors who are more likely to have G6PD deficiency. This review discusses G6PD deficiency, its importance in transfusion medicine, changes in the RBC antioxidant system (of which G6PD is essential) during refrigerated storage, and mechanisms of hemolysis. In addition, as yet unanswered questions that could be addressed by translational and clinical studies are identified and discussed.

Keywords

glucose-6-phosphate dehydrogenase deficiency; transfusion; oxidative stress; sickle cell disease; hemolysis

INTRODUCTION

Approximately 400 million people worldwide are glucose-6-phosphate dehydrogenase (G6PD) deficient making it the most common human enzyme deficiency. G6PD catalyzes the first step in the pentose phosphate pathway (equivalently, the hexose monophosphate shunt) to generate NADPH which is subsequently utilized in processes that reduce hydrogen peroxide (H₂O₂) to water, ameliorating oxidative stress in red blood cells (RBCs).

In 1956, G6PD deficiency was first described in the setting of hemolysis induced by the anti-malarial medication primaquine.[1] Subsequently, fava bean ingestion, various infections, certain oxidative medications, and some herbal remedies were associated with hemolysis in G6PD-deficient individuals. The prevalence of G6PD deficiency varies among ethnic groups with overall lower frequency in the Americas (3.4%), Europe (3.9%), and the

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Pacific (2.9%) as compared to sub-Saharan Africa (7.5%), the Middle East (6.0%), and Asia (4.7%).[2]

The *G6PD* gene is located on the X-chromosome. It is generally believed that G6PD-deficient RBCs are unable to generate sufficient NADPH when exposed to oxidative stress, leading to inadequate availability of reduced glutathione (GSH), and lack of protection against reactive oxygen species. These reactive oxygen species induce protein and lipid peroxidation, thereby causing intravascular RBC lysis and/or extravascular RBC clearance by macrophages in the reticulo-endothelial system.

G6PD-deficient RBCs also respond less well to oxidative stress induced by refrigerated storage,[3] with decreased recovery *in vivo* after transfusion.[4] Several case reports describe hemolysis *in vivo* following transfusion of G6PD-deficient RBCs,[5-9] thereby raising the question of whether it is safe to transfuse RBCs from G6PD-deficient donors. However, information regarding possible adverse effects of transfusing G6PD-deficient RBCs remains limited. Therefore, blood donors are not routinely screened for G6PD deficiency, and blood center policies differ regarding deferral of known G6PD-deficient donors.

Certain RBC transfusion-dependent patients may be at greater risk of receiving blood from G6PD-deficient donors. For example, sickle cell disease (SCD) patients often require transfusion of blood group antigen-negative RBCs to prevent or respond to alloantibody formation; therefore, based on blood group antigen frequencies, they are more likely to receive RBCs from donors of African descent who are also more likely to be G6PD deficient.[10] This would place these patients at increased risk if they developed an infection, or if they simultaneously received a medication that induced oxidative stress (e.g. a pregnant SCD patient treated with nitrofurantoin for a urinary tract infection).

With the changing recommendations for transfusion and treatment regimens for specific patient populations, the relevance of G6PD deficiency in blood donors is increasing, and warrants renewed attention. In an effort to protect transfusion recipients without needlessly deferring blood donors, this review discusses the relevance of G6PD deficiency to transfusion medicine and identifies unanswered questions on this topic.

PHYSIOLOGY OF G6PD

The RBC antioxidant system has many components, including peroxiredoxins,[11] superoxide dismutase, catalase, GSH, methemoglobin reductase, and vitamin E. NADPH is the key source of reducing equivalents, and G6PD acts on glucose-6-phosphate to produce NADPH and 6-phosphogluconate (Fig 1). In RBCs, G6PD is essential for maintaining the NADPH supply, which is used by glutathione reductase to reduce oxidized glutathione (GSSG) to GSH, making it central to GSH production. In addition, NADPH maintains catalase in its active form,[12] allowing it to catalyze the conversion of H_2O_2 to H_2O and O_2 . While it is not the primary methemoglobin reductase, NADPH-methemoglobin reductase also uses NADPH to reduce the Fe^{+3} in methemoglobin to Fe^{+2} , thereby allowing hemoglobin to bind oxygen. GSH is a critical reducing agent in multiple reactions, thereby managing the oxidative state of RBC proteins and lipids. Finally, glutathione peroxidase converts H_2O_2 to H_2O in a GSH-dependent reaction. Therefore, NADPH production by G6PD is critically important for the proper functioning of the RBC antioxidant system.

The *G6PD* gene is located on the long arm of the X-chromosome (Xq28),[13] near the genes for Factor VIII and color blindness. It is composed of 13 exons and 12 introns, and encodes a 515 amino acid monomer, with homo-dimers or -tetramers comprising the active form of the enzyme.[14, 15] The wild-type form of *G6PD* is termed “*G6PD B*”. Because mature

RBCs lack nuclei, they do not continue to express the *G6PD* gene; therefore, as RBCs age *in vivo*, G6PD activity decreases,[16] probably due to protein instability. Therefore, measuring G6PD shortly after a hemolytic event, during brisk reticulocytosis, may over-estimate its true activity.

G6PD status can be assessed by measuring enzyme activity, protein expression, and/or genotype. For routine clinical purposes, enzyme activity is determined by measuring G6PD-catalyzed NADPH production from NADP⁺. Quantitative G6PD assays measure the rate at which NADPH is produced when an aliquot of RBC lysate (which contains G6PD) is added to a reaction mixture containing the relevant enzyme substrates (e.g. glucose-6-phosphate and NADP⁺). NADPH production is measured spectrophotometrically at 340 nm, and enzyme activity is normalized by expressing it as one of the following ratios: (activity)/(hemoglobin concentration) or (activity)/(RBC number).

Qualitative and semi-quantitative G6PD assays also measure NADPH generation, but do not require an ultraviolet spectrophotometer. For example, NADPH fluoresces, in contrast to NADP⁺, and this is the basis of the fluorescent spot test.[17] Other such tests indirectly determine NADPH production by various methods.[18] In addition, Heinz body detection[17] and GSH stability[19] can identify G6PD-deficient individuals. Semi-quantitative assays are commonly used for screening because they are simpler to perform than quantitative tests. However, they may not correctly diagnose female heterozygotes, whose G6PD levels are often not low enough to be detected by these tests; genetic testing is the best approach for definitive diagnosis in this setting.[20]

Because G6PD deficiency is X-linked, males are more commonly affected than females. Due to X-inactivation (i.e. “lyonization”), heterozygous females are genetic mosaics; therefore, their degree of G6PD deficiency and their resulting phenotype are more variable. In contrast, hemizygous males and homozygous females (or compound heterozygous females having two deficient variants) with defective *G6PD* alleles tend to be functionally G6PD deficient.

There are approximately 200[21] mutations and 400 biochemical variants of G6PD. Biochemical variants are classified based on enzyme activity, electrophoretic mobility, Michaelis constant, thermostability, utilization of substrate analogues, and clinical phenotype. The World Health Organization (WHO) classifies G6PD variants by enzyme activity and association with chronic non-spherocytic hemolytic anemia (CNSHA). Class I comprises severely deficient (<1% activity) variants that are associated with CNSHA. Class II variants have <10% activity and are not associated with CNSHA. Class III variants have 10-60% enzyme activity, Class IV variants have 60-150% enzyme activity (normal), and Class V variants have increased enzyme activity.[22] The mutations underlying G6PD variants are typically missense point mutations resulting in amino acid substitutions. Small in-frame deletions are rarely identified; large deletions and rearrangements have not been found. Mutations inducing the more severe phenotype associated with CNSHA tend to be clustered in exons 6, 10, and 13, which encode the regions for substrate binding, the dimer interface, and the structural NADP⁺ site, respectively. Class II and III variants tend to have mutations distributed throughout the gene.

CLINICAL PRESENTATION

The clinical spectrum of G6PD deficiency varies. Patients with the most severe mutations, Class I, suffer from chronic hemolysis. Patients with Class II and III deficiency do not have chronic hemolysis, but may experience episodes of hemolysis due to infection or exposure to a substance causing oxidative stress. Individuals with the Mediterranean variant (Class II) have a shortened RBC lifespan at steady state (Cr⁵¹ half-life shortened from 32.0 days in

control subjects to 28.9 days in deficient subjects),[23] along with decreased baseline hemoglobin levels and increased reticulocytes;[24] favism (i.e. hemolysis following fava bean ingestion) occurs in these individuals. Conflicting data exist concerning RBC lifespan in individuals with the A- variant (Class III), with some demonstrating shortened[25] or normal[26] lifespan. Hemolysis following drug administration tends to develop with a 1-2 day delay, and is usually self-limiting in patients with the A- variant,[26] but more severe in patients with the Mediterranean variant.[27] Laboratory findings in G6PD-deficient patients undergoing a hemolytic event can include decreased hemoglobin and hematocrit, decreased haptoglobin, increased lactate dehydrogenase, unconjugated hyperbilirubinemia, methemoglobinemia, hemoglobinuria, and a negative Direct Antiglobulin Test. The peripheral smear may show hemi-ghosts (i.e. RBCs with hemoglobin sequestered to only half of the cell) and Heinz bodies.

GEOGRAPHIC DISTRIBUTION

The prevalence of G6PD deficiency varies by geographic location. Overall, the distribution of G6PD-deficient individuals correlates with the global distribution of malaria, and this enzymopathy may protect against malaria infection.[28] Consistent with this association, G6PD deficiency is most common in sub-Saharan Africa, the Middle East, the Mediterranean, and Southeast Asia.[2] G6PD-deficient RBCs, because of their enhanced sensitivity to oxidant stress, may provide a hostile environment for *Plasmodium* species, causing oxidative damage to the parasites and their subsequent destruction.[29] In addition, malaria-infected G6PD-deficient RBCs are ingested by macrophages at earlier stages of parasite maturation than infected wild-type RBCs.[30]

Although G6PD variants exist in multiple populations, some variants are found in specific ethnic groups.[31] For example, the most common variant, G6PD A-, accounts for most cases of G6PD deficiency in sub-Saharan Africa and in areas populated by people of African descent (e.g. North America, the Caribbean). In the United States, males of African descent have the highest frequency of G6PD deficiency, with a prevalence approaching 10%.[32] The next most common variant is G6PD Mediterranean, which is prevalent around the Mediterranean Sea (e.g. Greece, Italy).

MODELS OF G6PD DEFICIENCY

Experimental models of G6PD deficiency *in vivo* and *in vitro* use both chemical treatments of normal RBCs as well as animal models. For example, G6PD deficiency results in the failure to produce sufficient NADPH, leading to decreased GSH and the inability to generate GSH in response to oxidative stress. Therefore, treating normal RBCs *in vivo* or *in vitro* with phenylhydrazine mimics the G6PD-deficient state by increasing levels of reactive oxygen species, increasing lipid and protein peroxidation, and depleting intracellular GSH levels.[33] Similar results occur when treating animals with divicine[34] (i.e. the active hemolysis-producing agent in fava beans) and dapsone;[35] in particular, animals treated with these substances exhibit hemolysis.[36] In addition, treating cells with agents such as dehydroepiandrosterone (DHEA),[37] aldosterone,[38] and colchicine,[39] inhibits G6PD activity. Although G6PD activity varies in RBCs obtained from different inbred mouse strains,[40] no clinical phenotype or difference in GSH stability is reported. In contrast, Pretsch *et al.* isolated mice with X-linked G6PD deficiency (with a normal G6PD protein produced in reduced quantity) from the offspring of male mice treated with 1-ethyl-1-nitrosurea. Hemizygous males and homozygous females have G6PD activity that is approximately 15% of normal, whereas heterozygous females have 60% activity.[41] Attempts to create female mice with homozygous *G6PD* deletion (i.e. “knock out” mice)

were unsuccessful because of embryonic lethality;[42] interestingly, no human patients have been identified, to date, with a complete absence of G6PD activity.

MECHANISMS OF RBC DESTRUCTION

The clinical manifestations of G6PD deficiency include CNSHA, neonatal hyperbilirubinemia, favism, and hemolytic anemia induced by drugs, dietary supplements, or infection. When G6PD-deficient RBCs are exposed to primaquine, or when normal RBCs are treated with phenylhydrazine, hemolysis is primarily extravascular, due to macrophage ingestion in the spleen and liver; nonetheless, intravascular hemolysis also occurs.[43] For example, in chickens, massive erythrophagocytosis by Kupffer cells follows phenylhydrazine injection.[44] In addition, phagocytosis of human G6PD-deficient RBCs and phenylhydrazine-treated normal human RBCs by mouse macrophages (*in vitro*) involves Fc and lectin-like receptors as demonstrated by decreased phagocytosis in the presence of Fc receptor blocking agents as well as pre-incubation of the macrophages with galactose and mannose.[45, 46] Autologous antibodies have been detected on the surface of RBCs and may undergo conformational/topological changes in response to oxidative stress, resulting in Fc receptor-mediated recognition and phagocytosis of the RBCs by the macrophages.[45] Ingestion of oxidatively-damaged RBCs by Kupffer cells in the liver also involves the recognition of exposed phosphatidylserine by scavenger receptors.[47] It has also been demonstrated that reduction in the RBC effective surface area caused by membrane cross linking (resulting in the formation of hemi-ghost RBCs) during hemolytic crises may increase sequestration of damaged RBCs in the reticulo-endothelial system leading to increased phagocytosis and extravascular hemolysis. Decreased effective RBC surface area may also cause these RBCs to undergo osmotic lysis.[48]

Medication- and Food Exposure-Related Hemolysis

Although numerous drugs have been associated with hemolysis in G6PD-deficient patients, [49] a recent evidence-based review identified dapsone, methylene blue, nitrofurantoin, phenazopyridine, primaquine, rasburicase, and toluidine blue as the most important, commonly used drugs that should be avoided.[50] Studies *in vitro* have been used to predict the potential of various agents to cause hemolysis in G6PD-deficient individuals. Although these experiments provide relevant insights, it is important to note that, in some cases, it is not the drug or agent itself that causes hemolysis, but rather a metabolite; for example, this was elegantly shown with dapsone.[51] In addition, inter-individual differences in drug metabolism, such as the acetylator phenotype, may play a role.[52] Finally, some medications or dietary agents may only be associated with hemolysis in patients with certain G6PD variants.[31]

Apart from the widely-cited medications associated with hemolysis in G6PD-deficient individuals, hemolysis also occurs following exposure to other agents, such as naphthalene, [53] often in the form of ingestion or inhalation of moth balls. In addition, hemolysis occurs following the use of herbal medicines/remedies. For example, henna is associated with hemolysis following its topical application, particularly in G6PD-deficient newborns.[54] In this case, the active ingredient responsible for RBC destruction is identified as lawsone (2-hydroxy-1,4 naphthoquinone). In South-East Asia, hemolysis occurs following ingestion of *Acalypha indica*. [55] In Chinese herbal medicine, preparations containing berberine are associated with hemolysis in G6PD-deficient newborns; [56] this association with hemolysis, and even death, prompted the government of Singapore to ban berberine as a component of legal herbal medicines. In studies *in vitro*, berberine depletes GSH in RBCs from G6PD-deficient individuals.[57] Finally, acute hemolysis occurred in G6PD-deficient individuals taking high doses of ascorbic acid; [58] studies *in vivo* and *in vitro* reveal that, although normal ascorbic acid levels protect against oxidative stress, [59] ascorbic acid in large doses

decreases the function and survival of G6PD-deficient RBCs.[60] Ascorbic acid functions as an antioxidant at physiologic concentrations through its donation of an electron to oxidizing radicals. Pharmacologic doses of ascorbic acid, however, promote the production of H_2O_2 as a by-product of cycling between the ionized ascorbic acid and the ascorbate radical,[61] resulting in damage to G6PD-deficient RBCs.

Infection-Related Hemolysis

Hemolysis in G6PD-deficient individuals is commonly induced by infection.[53] Indeed, some argue that many incidents of hemolysis thought to be induced by medications should actually be attributed to the presence of fever and/or infection. Both viral and bacterial infections are associated with episodes of hemolysis.[53] Importantly, numerous cases of severe hemolytic events, which led to acute renal failure and even death, are associated with infection due to hepatitis A[62] and E viruses.[63] Possible mechanisms for the association of such severe hemolytic events with hepatitis infection include observations that RBC GSH becomes depleted during hepatitis,[64] and this would be expected to be more profound in G6PD-deficient RBCs. In relation to hemolysis associated with bacterial infections, the role of the oxidative burst and release of H_2O_2 by granulocytes was investigated. To this end, G6PD-deficient, but not normal RBCs have decreased GSH when incubated with activated neutrophils, and GSH depletion requires the neutrophils and RBCs to be in close proximity to one another. This process may be complement dependent, involving immune complexes that physically link RBCs to neutrophils and bacteria.[65] This scenario may be particularly relevant in the spleen of an infected individual, in which RBCs come into close contact with phagocytes and immune complexes. Finally, in a mouse model of polymicrobial sepsis, decreased RBC deformability, increased circulating free hemoglobin, and GSH depletion were all seen in G6PD-deficient mice.[66]

Diabetes-Related Hemolysis

Diabetic ketoacidosis (DKA) is thought to cause hemolysis in G6PD-deficient patients. One factor that may be responsible is decreased GSH levels in the blood of diabetic patients,[67] making G6PD-deficient RBCs more susceptible to oxidative stress. In contrast, another study suggested that DKA was not a major cause of hemolysis in G6PD-deficient patients because most of these cases also involved medications and/or infections as plausible causes of hemolysis.[68] Importantly, in many cases of hemolysis associated with DKA, the hemolysis usually occurs following correction of blood glucose levels into the euglycemic range.[69] For example, in one diabetic patient, repeated episodes of hemolysis occurred with each hospital admission, coinciding with iatrogenic hypoglycemia after insulin administration; further hemolysis was prevented by maintaining his blood glucose in the high-normal range.[70] One possible explanation for the induction of hemolysis following the return to normoglycemia is that, during hyperglycemia, there is an excess of substrate (i.e. glucose-6-phosphate), which allows for NADPH production, even with the reduced G6PD activity in G6PD-deficient patients. When glucose returns to normal or low levels, this sudden decrease leads to an abrupt decrease in NADPH production and a consequent decrease in GSH, thereby inducing hemolysis. Therefore, high blood glucose levels in G6PD-deficient patients with DKA should be corrected carefully, and they should be observed for hemolysis.

TRANSFUSION MEDICINE AND CELLULAR THERAPY

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell (HSC) transplantation may involve a G6PD-deficient recipient or donor. For G6PD-deficient recipients, it is important to note that medications used for hematologic malignancies, including chemotherapeutic agents and rasburicase, can cause

hemolysis in G6PD-deficient patients.[31, 50] In addition, following transplant, trimethoprim-sulfamethoxazole is typically used for prophylaxis against *Pneumocystis jirovecii* pneumonia. In patients who are unable to tolerate trimethoprim-sulfamethoxazole, dapsone is often used. Because both of these medications can cause hemolysis in G6PD-deficient patients, pentamidine may be substituted.

HSCs (from peripheral blood,[71] bone marrow,[71] and umbilical cord blood[72]) from G6PD-deficient donors have been successfully used for transplantation. Although data are limited, no adverse events related to granulocyte colony stimulating factor treatment are reported in G6PD-deficient stem cell donors.[71] In long-term follow-up studies of recipients of HSCs from G6PD-deficient donors, there were no decreases in engraftment or survival, as compared to recipients of HSCs from normal donors.[71, 73] In most cases, the recipient converts to the G6PD status of the donor;[73] therefore, it is important to be aware that, following engraftment, a recipient of an HSC graft from a G6PD-deficient donor will also be G6PD deficient. This observation leads some to advocate the use of serial measurements of G6PD activity following transplantation as an indicator of engraftment status.[72] Additionally, when the HSC donor is a G6PD heterozygous female, the extent of G6PD deficiency in the recipient following engraftment can differ from that in the original donor, due to possible skewing of X-inactivation at the time of engraftment. Therefore, some authors recommend repeat G6PD screening approximately 6 months after transplantation for recipients of HSCs from female donors.[74]

RBC Transfusion

The “RBC storage lesion” refers to biochemical and biophysical changes in RBC physiology that occur during refrigerated storage.[75] These include decreased pH, depletion of 2,3-diphosphoglycerate and ATP, increased extracellular lactate and potassium, decreased RBC deformability, increased RBC fragility, and accumulation of RBC microvesicles,[76] all of which may contribute to hemolysis *in vitro* and *in vivo*. RBC storage under conventional conditions is considered an oxidative stress for RBCs, as evidenced by increases in reactive oxygen species over time,[77] accumulation of oxidative biomarkers, including malondialdehyde[78] and oxidation products of human serum albumin[79], as well as oxidation-dependent alterations of RBC membrane proteins, such as spectrin.[80] Further evidence implicating oxidative stress in the adverse effects of storage on RBCs include improvements in the storage lesion, in post-transfusion survival,[81] and in storage-related protein processing,[82] when RBCs are stored under anaerobic conditions, when antioxidants are administered to blood donors prior to donation,[83] and when blood is stored in the presence of antioxidants.[84]

Studies have examined the activity of the RBC antioxidant system during storage. Because G6PD activity decreases as RBCs age *in vivo*,[16] the question was raised as to whether the same phenomenon occurs during refrigerated storage. Studies of G6PD activity during RBC storage under standard blood banking conditions show a range of results, with some demonstrating no decrease in G6PD activity[10, 85] or activity decreases of up to 35%.[78] The reasons for these differences are not clear; however, methodological variations in storage conditions and preservative solutions may play a role. GSH levels decrease during RBC storage[78] and, importantly, GSH stability and stimulated pentose phosphate pathway activity also decrease.[3] Taken together, these data suggest that there are alterations in RBC antioxidant mechanisms during storage, and the effects of these changes may be most notable when an additional oxidant challenge is present. Although G6PD activity of stored RBCs is not affected by gamma irradiation,[86] irradiated G6PD-deficient RBCs have a significantly shortened half-life, as compared to normal irradiated RBCs.[87]

The adverse consequences of transfusing RBCs from G6PD-deficient donors may vary based on the clinical status of the recipient, as well as on the types of mutations in the donor population. Blood donors are not routinely screened for G6PD deficiency and blood centers differ in their deferral of donors with known G6PD deficiency; that is, some defer if a donor reports being G6PD deficient, whereas others do not have a policy for such donors. Using two methods for assessing RBC survival, Brewer *et al.* showed that the half-life of G6PD-deficient RBCs is significantly shorter than that of normal RBCs, even in the absence of administering an oxidizing drug.[25] In addition, the 24-hour post-transfusion recovery of autologous G6PD-deficient RBCs following refrigerated storage is significantly less than that for autologous normal RBCs.[4] Thus, G6PD deficiency may provide one genetic reason for the occurrence of donors whose RBCs store poorly.[88] Nonetheless, these classical papers involved relatively few subjects, suggesting that studies of post-transfusion recovery of G6PD-deficient RBCs stored using current standards would be useful.

Extravascular and intravascular hemolysis, as well as decreased RBC recovery, were reported in subjects transfused with RBCs from G6PD-deficient donors when the recipients were treated with a hemolysis-inducing drug.[43] In several case reports, patients experienced unexplained hemolysis after RBC transfusion. Following investigation the most likely cause of hemolysis was the transfusion of blood from G6PD-deficient donors.[5-9] In the most dramatic case, a patient who was simultaneously being treated for leprosy with dapsone, experienced profound hemolysis leading to organ failure and death.[9] Indeed, such cases can be misclassified as an antibody-mediated transfusion reaction. In addition, neonatal exchange transfusion with G6PD-deficient RBCs may be associated with higher post-transfusion total bilirubin levels than exchange transfusion with normal RBCs.[89] Other studies evaluated patients who, although not treated with oxidative medications, were transfused with RBCs from G6PD-deficient donors. In one such study, patients were transfused with blood from donors who had either undetectable or normal G6PD activity; however, there were no statistically significant differences in post-transfusion hemoglobin, hematocrit, bilirubin, or haptoglobin levels in these two groups of recipients.[90] Similarly, McCurdy and Morse did not note serious adverse events following transfusion of G6PD-deficient RBCs from donors with the Class III A- variant.[91] The authors state, however, that follow up of some of the transfusion recipients was not complete and that two subjects who had also received 1,000 mg of intravenous ascorbic acid either had suboptimal increase in hematocrit, or an accelerated decrease in hematocrit while receiving this therapy.[91] Table 1 summarizes the published reports concerning the outcomes of patients transfused with blood from G6PD-deficient donors. Taken together, these results suggest that adverse events may occur after transfusion of G6PD-deficient RBCs, particularly in patients concurrently receiving certain medications. Importantly, accelerated destruction of G6PD-deficient RBCs, either in the storage bag or following transfusion may result in increased levels of extracellular hemoglobin. Hemolysis-associated pathologies including smooth muscle dystonia, vasculopathy, and endothelial dysfunction are believed to be caused by nitric oxide scavenging by cell-free hemoglobin.[92]

The results discussed above raise the question of whether it is necessary to screen blood donors for G6PD deficiency. Based on cost, it is unclear if it is advisable to perform an additional test on donors without a definite benefit to recipients. Because the prevalence of G6PD deficiency differs among populations, this also affects its prevalence among donors, depending on their racial/ethnic composition. For example, the reported frequency of G6PD deficiency among blood donors varies significantly: Nigeria (19.5%),[93] Canada (0.17%), [94] Brazil (3.2%),[95] Spain (0%),[96] Iran (16.3%),[97] India (0.8%),[98] Sardinia (6.6%),[99] Yemen (7.1%),[100], Saudi Arabia (10.7%),[101] Kuwait (6.5%),[102] and the United States (0.3%).[10] Among G6PD-deficient donors in Yemen and Kuwait 97.2% and 80%, respectively, had Class II (<10% activity) variants with the majority being the

Mediterranean variant.[100, 102] Although most G6PD-deficient individuals in sub-Saharan Africa and the Americas (>80% in Brazil and Central America) are expected to have the Class III A- variant[103] which has been termed “mild”, it is important to note that severe hemolysis following treatment with dapsone[104] as well as favism[105] have been observed in individuals with this variant. Because of the variation in prevalence, donor screening could be targeted to populations with increased G6PD prevalence. In addition, donors need only be tested once for G6PD deficiency because of its genetic inheritance. Finally, it may not be necessary to screen donors at collection; rather, if the RBCs were to be transfused into a recipient under oxidative stress (e.g. infection or treatment with an oxidative medication), the donor RBCs could be tested just before release for transfusion. The WHO suggests that policies for the assessment of prospective donors should be developed in regions where there is a high incidence of RBC enzymopathies such as G6PD deficiency and inherited membrane defects. According to the WHO Guidelines on Assessing Donor Suitability for Blood Donation, individuals with G6PD deficiency who do not have a history of hemolysis may be accepted for donation, however, blood from these donors should not be used for intrauterine transfusion, neonatal exchange transfusion, or for G6PD-deficient patients. In addition the WHO recommends deferring G6PD-deficient donors with a history of hemolysis.[106] Standards in the United States[107] and the United Kingdom[108] do not offer specific guidance about G6PD-deficient blood donors.

Chronically-transfused SCD patients have relatively high rates of alloimmunization to blood group antigens.[109] Because of the need for antigen-negative compatible units, they may be more likely to receive RBCs from donors of African descent, who have a high prevalence of G6PD deficiency. A study of 10,000 consecutive male donors in a New York City hospital blood bank demonstrated that 13% of donors of African descent were G6PD-deficient versus a frequency of 0.9% in Caucasian donors.[53] We recently found that the frequency of G6PD deficiency among random RBC units in a metropolitan transfusion service (0.3%) was significantly lower than that of units used for exchange transfusions for SCD patients receiving Rh-positive units that were negative for the C and E antigens (12.3%).[10] Thus, with the growing popularity of programs that provide phenotypically-matched RBCs for SCD patients, the possibility of receiving a G6PD-deficient unit may be higher for these patients. In addition, if the frequency of G6PD-deficient units in the inventory of our transfusion service (0.3%) is generalizable to other areas within the United States, since ~15 million RBC units are transfused each year in the United States,[110] ~45,000 units of G6PD-deficient RBCs are potentially transfused annually. Systematic assessments of appropriate physiologic and laboratory parameters are necessary to fully evaluate the impact this has on patient outcomes.

UNANSWERED QUESTIONS

Many unanswered questions remain about the safety of transfusing RBCs obtained from G6PD-deficient donors. These suggest additional studies that can be performed *in vitro*, with animal models *in vivo*, and with human blood donors and transfusion recipients. Some examples are provided below:

1. Does the nature of the RBC storage lesion differ between G6PD-deficient and normal RBCs? In addition, is it more severe for G6PD-deficient RBCs?
2. What are the differences in antioxidant response between normal and G6PD-deficient RBCs following refrigerated storage?
3. Are there improved methods of maintaining the RBC anti-oxidant system during refrigerated storage?

4. Using current RBC storage techniques (e.g. pre-storage leukoreduction, preservative solutions), does post-transfusion RBC recovery *in vivo* differ between G6PD-deficient and normal RBCs?
5. Which laboratory results and physiologic outcomes are associated with transfusions of G6PD-deficient RBCs in steady-state recipients and in those under oxidative stress (e.g. with infection or while receiving particular medications)?
6. If refrigerator-stored G6PD-deficient RBCs are of lower “quality,” does this vary based on the underlying G6PD genetic mutation?
7. What are the costs and benefits of screening for G6PD-deficiency in the general blood donor population, in select donor populations, or at the point-of-care in a hospital transfusion service?
8. Do genetic or environmental conditions that affect the RBC antioxidant system, other than G6PD-deficiency, affect RBC storage quality?

CONCLUSIONS

In summary, G6PD deficiency is the most common enzymatic abnormality worldwide, but its importance in transfusion medicine is relatively understudied and under-appreciated. Blood donors are not routinely screened for G6PD deficiency, which may lead to adverse consequences if G6PD-deficient RBCs are transfused into a susceptible host (e.g. during infection or treatment with an oxidative drug). Increased blood group antigen matching for SCD patients, with concomitant increased exposure to transfusions with G6PD-deficient RBC units, suggests that this warrants additional attention. Thus, in certain populations, the biochemistry of antigens on the RBC cell surface is genetically linked to enzymes responsible for metabolic pathways in the RBC cytosol. Through well-designed, ethical studies employing experiments *in vitro*, in animal models, in human blood donors, and in transfused patients, the transfusion medicine community can better assess the potential risks associated with G6PD-deficiency and RBC transfusion.

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Figure 1.

Glucose-6-phosphate dehydrogenase in the pentose phosphate pathway. In response to oxidative stress, superoxide dismutase (SOD) forms H₂O₂ from the superoxide anion (O₂⁻). Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the first step of the pentose phosphate pathway using glucose-6-phosphate (G6P), formed as a result of phosphorylation of glucose by hexokinase (HK), and NADP⁺, to produce NADPH and 6-phosphogluconate (6PG). NADPH acts as an electron donor in the reduction of oxidized glutathione (GSSG) by glutathione reductase (GR) to produce reduced glutathione (GSH). H₂O₂ is reduced to water by either glutathione peroxidase (GPx) or catalase, the latter of which also utilizes NADPH for its enzymatic function.

Table 1

Published reports involving transfusion of G6PD-deficient RBCs.

Patients	Transfusion	Oxidative Medication?	Outcome	Ref
23 patients	Simple	No	No hemolysis	[91]
48 patients	Simple	No	No hemolysis	[90]
19 infants	Exchange	No	No hemolysis	[111]
10 patients	Simple	No	bilirubin & LDH	[8]
1 patient	Simple	No	bilirubin & LDH	[7]
2 infants	Exchange	No	Prolonged bilirubin	[112]
21 infants	Exchange	No	Slower bilirubin than controls	[89]
1 neonate	Exchange	No	HCT, bilirubin, hemoglobinuria	[6]
1 neonate	Exchange	No	HCT, bilirubin, hemoglobinuria	[5]
37 patients	Exchange & Simple	No	HCT, bilirubin, hemoglobinuria	[113]
1 patient	Simple	Yes (dapsone)	Hemolysis, organ failure, death	[9]

Abbreviations: HCT, hematocrit; LDH, lactate dehydrogenase.