Case study

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY CO-INCIDENT WITH AUTOIMUNE HAEMOLYTIC ANAEMIA IN AN ADOLESCENT FEMALE: A CASE REPORT.

ABSTRACT

Background: Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is a genetic metabolic abnormality caused by deficiency of G6PD an enzyme critical for the proper function of red blood cells. The *G6PD* gene mutation is inherited in an X-linked recessive manner affecting males and heterozygous females with unfavorable X-inactivation (lyonized females). Autoimmune hemolytic anemia (AIHA) is characterized by a shortened red blood cell (RBC) survival and presence of autoantibodies directed toward autologous RBCs, usually demonstrated by a positive direct antiglobulin (Coombs) test (DAT). AIHA may be sub-classified into *primary* or *idiopathic* and *secondary based on* the absence or presence of an underlying disease. Secondary forms are common in **teenagers**. For instance an increased incidence of autoimmune phenomenom has been reported in female X-linked carriers of the chronic granulomatous disease (CGD) gene. Additionally, homozygous G6PD deficient females with coincidence of a rare immune disorder termed chronic granulomatous disease (CGD) have been described. We report a case of a G6PD deficient adolescent female with fever, steroid-responsive haemolytic anaemia and positive direct Coombs test.

Keywords: AIHA, G6PD deficiency, CGD, Adolescent female, steroid-responsive.

INTRODUCTION

G6PD deficiency is a genetic metabolic abnormality caused by deficiency of G6PD an enzyme critical for the proper function of red blood cells (RBC). The *G6PD* gene is located on the long arm (q) of the X chromosome (Xq28) of RBC and neutrophils[1] The precise mutation is known for 186 of the 400 reported genetic variants[2]. The enzyme has been classified into I to V in

order of increasing enzyme activity and decreasing disease severity. In many cases mutation occurs as a new (sporadic or de novo) mutation. In cases with a family history, the *G6PD* gene mutation is inherited in an X-linked recessive manner affecting males and heterozygous females with unfavorable X-inactivation (lyonized females) [3,4].

The *G6PD* catalyzes the formation of coenzyme NADPH, which protects cells from oxidative damage. A mutation in the *G6PD* gene results in low levels of functional G6PD, which in turn leads to low levels of NADPH and a depletion of an antioxidant known as glutathione, which is necessary to protect the cell's hemoglobin and its cell wall from highly reactive oxygen radicals[3]. This reduction in NADPH makes red blood cells more susceptible to destruction from oxidative stress than other cells. When the level of this enzyme is too low and in the presence of "trigger" factors red blood cells can break down prematurely (hemolysis).Triggers of hemolysis in G6PD-deficient persons include certain infectious diseases, certain drugs such as anti-malarials derived from quinine, moth balls (naphthalene) and eating fava beans(favism). In the absence of trigger factors most people with G6PD deficiency are normal. However, within 2-3 days after exposure to the trigger acute hemolytic anemia (AHA) develops. When the body cannot compensate for accelerated loss, anemia develops. Features include fatigue, pale color, jaundice even in the neonate (neonatal jaundice), shortness of breath, rapid heartbeat, dark urine, splenomegaly, heart failure, occasionally gastrointestinal symptoms. Most episodes, even severe ones, are usually self-limiting and resolve on their own. Individuals of all races and ethnic backgrounds are affected with varying severity, Africa being among those with the highest prevalence rates[5].

Case presentation

A diagnosis is based upon the identification of characteristic features via a thorough clinical evaluation, a detailed patient history, demonstrating decreased activity of the G6PD enzyme through either a quantitative assay or a screening test such as Beutler fluorescent spot tes[6]; a rapid and inexpensive test that visually identifies NADPH produced by G6PD under ultraviolet light. When the blood spot does not fluoresce, the test is positive; it is falsely negative in patients who are actively hemolysing. It can therefore only be done 2–3 weeks after a hemolytic episode. Elevated serum lactate dehydrogenase (a marker of hemolytic severity), reticulocytosis, heinz and bite cells on blood film are supportive.

Affected individuals do not require treatment. G6PD deficiency is often best managed by preventive measures, discontinuation of causative drug, treatment of the infectious trigger, IV fluids and blood in severe cases.

G6PD deficient homozygous females have been described in whom there is co-incidence of a rare immune disorder termed chronic granulomatous disease (CGD)[7,8,9]. CGD is an X recessive and Autosomal recessive inherited defective neutrophil function disorder caused by mutations of 1 of the genes that encode the proteins in the NADPH oxidase complex[9]. In CGD patients neutrophils are incapable of superoxide production, essential for the killing of fungi and catalase positive bacteria [8,9]. Activation of the NADPH-dependent oxidase requires stimulation of the neutrophils conversely effective neutrophil phagocytosis requires activation of NADPH-dependent oxidase[9]. A few individuals with apparent CGD have been described as having neutrophils deficient in G6PD activity. The erythrocytes of these patients also lack the enzyme; these patients have chronic hemolysis[9]. Complete absence of the enzyme has also been described [10]. CGD is a rare disease with an incidence of four to five per million individuals [9]. Approximately 65% of patients with CGD are males who inherit their disorder as

a result of mutations in the X-chromosome gene. The clinical presentation, attack rate and severity of infections are exceedingly variable. This is highlighted by Gray et al [10] among 3 male siblings of a Canadian family. The onset of clinical signs and symptoms may occur from early infancy to young adulthood. Granuloma formation and inflammatory processes are a hallmark of CGD, skin infections are among the most common illnesses encountered [9]. Female X-linked carriers of CGD have been reported with an increased incidence of autoimmune phenomen[7,11].

Autoimmune hemolytic anemia (AIHA) is characterized by the presence of autoantibodies directed toward autologous RBCs, usually demonstrated by a positive direct antiglobulin (Coombs) test (DAT) and destruction of the antibody coated RBC in the reticulo-endothelial system[12,13]. AIHA is classified by the temperature at which autoantibodies bind optimally to RBCs. In warm antibody AIHA hemolysis is mediated by antibodies (IgG) which bind to RBCs at 37 °C (98.6 °F) [14]. In cryopathic hemolytic syndromes, cold-reactive autoantibodies(IgM) [15] exhibit affinity for RBCs optimally at temperatures below body temperature. Unusual patients have mixed autoantibodies AIHA [15]. AIHA may be sub-classified into *primary* or *idiopathic* in the absence of an underlying disease and *secondary AIHA* when it occurs together and remits with correction of a suspect disease or when the suspect disease causes an immunologic aberration [16]. Secondary causes of AIHA include chronic lymphocytic leukemia (CLL), lymphomas, Systemic lupus erythematosus (SLE). Certain drugs also mediate immune injury to RBCs. Three mechanisms are recognized and two of these involve elements of autoimmunity, in which autoantibodies recognize and bind to specific RBC epitopes in concert with drugs or their metabolites or in which the autoantibody binds to RBC epitopes without the presence of drug[17].

Warm antibody AIHA constitutes about 80-90% of adult cases with an estimated annual incidence of one per 75,000-80,000 population in USA [16]. Annual incidence in Nigeria is unknown. Cold agglutinin disease accounts for about 10-20% of cases of AIHA being more common in women than in men with an estimated prevalence of 14 per million [16]. Primary warm antibody AIHA accounts for about half of the cases [16]. Warm antibody AIHA typically present with insidious onset of symptoms of anaemia. Less often a patient may note sudden onset features of severe anemia such as pallor, jaundice, hepatosplenomegaly, hyperpnea, tachycardia, angina, or heart failure over a few days. Symptoms and signs of the underlying disease are present in secondary AIHA. Idiopathic (primary) chronic cold agglutinin disease has its peak incidence after age 50 years while paroxysmal cold hemoglobinuria(PCH) which make up 2-5% and Donath-Landsteiner one-third of cases are most common in children[18]. Secondary forms are usually associated with B-cell lymphoproliferative disorder, adolescents or young adults with *Mycoplasma pneumoniae* infections, infectious mononucleosis and sometimes in children with chickenpox. Patients with idiopathic cold agglutinin disease have either a chronic hemolytic anemia or episodic, acute, self-limited hemolysis (as in *Mycoplasma pneumoniae* infections) with hemoglobinuria induced by chilling. The incidence of drug-induced immune hemolytic anemia is about one per million. The great majority result from the second- and third-generation cephalosporins, cefotetan, and ceftriaxone[17]. A careful history of drug exposure should be elicited. The clinical picture is also variable depending on the rate of hemolysis. Acute renal failure and death may accompany severe hemolysis.

Hematocrit levels range from less than 10% to compensated near-normal in warm antibody AIHA, mild to moderate anemia with hematocrit levels as low as 15-20% in chronic cold agglutinin disease, a rapid fall in hematocrit during a paroxysm in PCH and similar to those of

warm antibody AIHA or PCH in drug-induced immune hemolytic anemia depending on the mechanism of the drug-induced immune process [14,19]. Leukopenia, neutropenia, occasionally immune thrombocytopenia (Evans syndrome) have been reported in warm antibody AIHA [19]. During a paroxysm in PCH, leukopenia is noted early, followed by leukocytosis[18]. Hemolysis may lead to consumptive depletion of complement levels. The blood film is common to all types of AIHA and shows polychromasia representing reticulocytosis from the marrow. Spherocytes are frequently seen and in more severe cases, RBC fragments, nucleated RBCs, and erythrophagocytosis by monocytes may be seen. RBC autoagglutination may be seen in the blood film and in chilled anticoagulated blood from patients with cold-antibody AIHA. The DAT is positive with anti-IgG and negative with anti-C3 in idiopathic and drug-associated (alpha-methyldopa) warm AIHA, positive with anti-IgG and anti-C3 in patients with SLE-associated and idiopathic warm AIHA but rare in drug-associated cases and positive with anti-C3 but negative with anti-IgG in cold agglutinin disease also in warm antibody hemolytic anemia when the IgG antibody is of low affinity, in some drug-associated cases and in PCH [14]. The laboratory findings of drug-induced AIHA may resemble those of warm antibody AIHA or a cryopathic hemolytic syndrome, depending on the mechanism of the autoantibody induction and the target antigen [17]

In children warm antibody AIHA is self-limited following a rapid response to glucocorticoids. Infants and adolescents have an insidious onset with tendency to become chronic [20] in the older [21]. The mortality rate ranges from 10 to 30%, higher in those with chronic AIHA and in Evans syndrome [19,21]. Idiopathic cold agglutinin disease is benign, self-limited in post infectious type and PCH [18]. However, death may result from infection or severe anemia or sometimes from an underlying lymphoma. The Donath-Landsteiner antibody and PCH patients

may survive for years. Drug-induced immune hemolytic anemia is usually mild, and the prognosis is good with cessation of hemolysis and negative DAT shortly after the drug is discontinued and clears from the circulation but for cases of drug-induced autoantibodies which may remain positive for months.

Treatment of AIHA depends on the cause and may include corticosteroids, splenectomy, rituximab IV immune globulin, immunosuppressants, avoidance of blood transfusions (except in cases of life-threatening anemia), avoidance of triggers (eg, cold), and withdrawal of drugs.

We present a rare case of AIHA coexisting with G6PD deficiency in an adolescent female. She presented with complaints of paleness of the body, yellowness of eyes, generalized body weakness, high grade fever, abdominal pain and passage of cola-coloured urine. Respiratory rate and heart rate were significantly deranged >2SD from normal for age and sex. She had high grade pyrexia of 39 degree Celsius, was icteric, dyspneic, markedly pale, her spleen was not enlarged, she had lower abdominal tenderness and was having a normal menstruation. The patient had no such personal nor family history previously No causative medications was identified. Hematocrit was 9% and mean oxygen saturation was 90% in room air. Laboratory evidence was of intravascular haemolysis (peripheral blood film evidence of bone marrow erythroid hyperplasia but with no spherocytes, and haemoglobinuria). Blood group was ‘A’ Rh positive, Genotype was AA, G6PD was deficient as determined by the fluorescent spot method[6]. Other investigations carried out were complete blood count from the specimen in the EDTA bottle, and these were done by automation as described by Araoye and Luzattoo and showed normal platelet count, neutrophillic-leucocytosis. Blood film noted many band neutrophils but only mild toxic granulations. Retroviral screen was negative. Direct Coomb's test was positive for ‘warm’ antibodies. Nitrite positive urine analysis, few white blood cells and

normal red blood cells without cast were identified on urine microscopy probably due to the menstruation, malaria parasite was seen on blood film. Blood culture was not done. Abdomino-pelvic ultrasonography revealed mild enlargement of the kidney and spleen

She was immediately transfused with 'O' Rh positive sedimented red blood cells, intra-nasal oxygen, intravenous anti-malarial and antibiotic medications were given. Fever gradually resolved but haemolysis persisted with worsening hypoxaemia and progressively dropping hematocrit despite transfusion of additional 4 pints of 'A' Rh positive blood in daily aliquots.All blood unit were negative for hepatitis B and C antigen, HIV and VDRL but were not screened for G6PD deficiency. She responded to oral prednisolone as she became independent of blood transfusion and her haemoglobin rose by 2g/dl with no further fall. She was counseled.

Concerning her G6PD status. Hematocrit on discharge was 16% and patient's condition was stable. A week after discharge patient was still in stable clinical condition although with evidence of low grade haemolysis, she was commenced on tapering doses of prednisolone. A repeat G6PD status is planned as patient is still being followed-up at the time of this report.

DISCUSSION

G6PD deficiency is fairly common in Nigerian children while AIHA is rare; 15.3% overall prevalence of G6PD deficiency and 13 cases of AIHA (7 females and 6 males) over a 10 year period in Western Nigeria [12,22]. CGD is also a rare disease with an incidence of four to five per million individuals[9]. G6PD in Nigerian male and female children has been reported as 24.1% and 6.6% respectively, the Igbo children having the 2nd highest [22]. AIHA is also more common in males but has an adolescent female preponderance [23]. The index patient is an

adolescent female. While the precise incidence of AIHA is not known, the number of affected children (<20 years old) are estimated to be less than 0.2/100,000 with the highest rates seen in pre-school age children [23,24,25]. Thus AIHA can be seen throughout childhood, but of note should prompt more attention when seen in **teenagers** as they are more likely to have an underlying systemic illness[23], this seems to mirror our patient.G6PD has been reported in lyonized heterozygous or homozygous females [3], raising the suspicion in our patient. G6PD deficiency and AIHA are disorders associated with acute and or chronic haemolysis. Therefore, coincidence of both disorders could exacerbate haemolysis as evident in the index patient. The haemolytic episode in AIHA is usually preceded by an acute infection [26] suggested by the systemic inflammatory response syndrome with leucocytosis in our patient although a blood culture was not done to identify the causative organism.

While the absence of a history of previous haemolytic episode may imply an acute event the fact that the patient was stable with a haematocrit of 16% at discharge and follow-up suggest a compensated chronic low grade haemolysis. G6PD status was deficient despite been assayed at time of haemolysis when new RBCs would normally show normal activity. Intravascular haemolysis noted in this patient rather than extravascular support G6PD deficiency over AIHA. However the positive direct Coombs test and the mildy enlarged spleen on sonogram support extravascular haemolysis of AIHA. Moreover, absence of splenomegaly in 78% AIHA patients has previously been reported with median spleen size of 0.5 cm in secondary cases[28]. Mild toxic granuation and marked neutrophillia with the suggested degree of infectivity imply a defective neutrophil function as may be found in CGD.

This study agrees with the view that apart from males AIHA is also common in adolescent females who is likely to have an underlying illness [23]. In this instance AIHA being coincident

with G6PD deficiency raises the question of an existence of the rare, sporadic, chronic granulomatous disease (CGD) [9,10] with complete absence of G6PD and non-spherocytic haemolytic anemia[10]. Since G6PD deficient homozygous females with co-incident chronic granulomatous disease (CGD) have been described. While few individuals with apparent CGD have been described as having neutrophils and erythrocytes deficient in G6PD activity, these patients have chronic hemolysis[3,4]. Additionally, CGD patients have been reported with an increased incidence of autoimmune phenomena also in female X-linked carriers [11,28]. This study, having identified clinical and laboratory features of both AIHA and G6PD deficiency in an adolescent female creates the need for studies regarding the possible relationships between these two disorders in this scenario. For screening of CGD, the nitroblue tetrazolium (NBT) dye test or the more accurate **flow cytometry** test using dihydrorhodamine 123 (DHR) which detects oxidant production because it increases fluorescence when oxidized by H_2O_2 [29]. Molecular studies would also be useful in delineating the index patient as either a lyonized heterozygote or homozygous G6PD deficient female and a co-incident carrier of the CGD gene with autoimmune phenomena. These specialized tests could not be done in our patient due to financial constraint as well as unavailability of materials and personnel. Asymptomatic CGD patient has been reported[10]. The patient didn't have up to four criteria required for a diagnosis of SLE although AIHA has been reported to precede SLE as the only manifestation by months or years before serological confirmation [30]. Our patient had normal platelet count thereby ruling out the diagnosis of Evan's syndrome [31].

The study also shows that steroid therapy (prednisolone) is quite effective in acute AIHA while suggesting an AIHA induced by IgG rather than IgM [32,33]. Our patient's Coombs test also supported a warm reacting autoantibody but a monospecific antibody test was not done. It is

noteworthy that red cell transfusion could be useful in life-threatening anaemia, otherwise is rarely effective[26].

Although majority of cases of warm ALHA are acute and carry a better prognosis with the possibility of spontaneous resolution within 6 months, infants and adolesents are said to have a insidious onset with tendency to become chronic[19] the chronic being more difficult to treat.

Similar to Salawu and Durosinmi's report[12], our adolescent female patient had no history of chronic haemolysis but an insidious onset of symptoms with prompt response to glucocorticoids. She therefore needs monitoring for chronicity and a repeaat G6PD assay because of unscreened blood transfused. Prevalence of G6PD deficiency in blood donor having being reported as high in a part of the country.

CONCLUSION: C6PD deficiency and AIHA may co-exist in an adolescent female possibly as part of CGD but a definitive diagnosis of CGD is required.

LIMITATION: Failure to establish a definitive diagnosis of CGD, genetic anomaly or SLE.

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