

SJMLS - 10(1) - 035

Pathophysiology and Current Laboratory Investigations of Glucose-6-Phosphate Dehydrogenase Deficiency.

Ibrahim Kalle Kwaifa^{1*}, Osaro Erhabor¹, Abdulrahman Yakubu¹, Aliyu Ibrahim Bagudo³, Buhari Hauwa Ali¹, Onuigwe Festus Uchechukwu¹, Nura Muhammad Bunza², Mustapha Umar Kalgo³, Abdullahi Isiyaku³, Mukhtar Yeldu¹, Dahiru Isah¹, Moses Akila¹, Solomon Moses¹, Zainab Abubakar Ibrahim¹, Ukashat Almustapha¹, Muhammad Lubabatu Abbas¹, Adra Sumaiya Ibrahim¹, Stephen Cecilia¹, Ismail Usman Adenkunle¹, Nasiru Sadiq¹, and Muhammad Buhari¹.

Department of Haematology, School of Medical Laboratory Sciences, College of Health Sciences, Usmanu Danfodiyo University (UDU), Sokoto, North-Western, 2346, Nigeria ¹, Department of Medical Microbiology, School of Medical Laboratory Sciences, College of Health Sciences, Usmanu Danfodiyo University (UDU), Sokoto, North-Western, 2346, Nigeria ², Department of Immunology, School of Medical Laboratory Sciences, College of Health Sciences, Usmanu Danfodiyo University (UDU), Sokoto, North-Western, 2346, Nigeria ³.

Author for Correspondence*: Ibrahim.kwaifa@udusok.edu.ng/+234-703-548-394. <https://dx.doi.org/10.4314/sokjmls.v10i1.35>

Summary

Glucose-6-phosphate dehydrogenase catalyses the rate-determining step in the pentose phosphate pathway. Its activity is a key determinant of the reduced nicotinamide adenine dinucleotide phosphate to oxidized nicotinamide adenine dinucleotide phosphate (NADPH-to-NADP+) ratio in the cytoplasm and thus contributes to the replenishment of the antioxidant glutathione system. Glucose-6-phosphate dehydrogenase deficiency is a common X-linked human enzyme defect of red blood cells. Individuals with this gene defect appear normal until exposed to oxidative stress which induces haemolysis but it is infrequently taken into consideration in health practices. There is a need for the routine screening of subjects on a much wider scale for G-6-PD deficiency in our environment, especially in malaria endemic areas, where quinine and its derivatives are used. This would allow for evidence-based management of subjects with G-6-PD deficiency as well as educate them so as to avoid food, drugs, and other agents that can potentially predispose them to haemolytic crisis or oxidative stress. Data were collected from Taylor and Francis, PubMed, Springer, Nature, Google Scholar, MDPI, BMC and some other related data. In this review details on the molecular mechanisms, pathophysiology, laboratory investigation and possible therapeutic approaches for Glucose-6-Phosphate dehydrogenase deficiency were discussed.

Keywords: Pathophysiology, current laboratory investigation, G-6-PD and therapeutic approaches

1.0 Introduction

Every cell expresses the cytoplasmic enzyme G-6-PD, which is involved in the initial phase of the pentose phosphate pathway of glycolysis. Because it eliminates harmful oxygen radicals, this enzyme is essential for preventing premature erythrocytes lysis and protecting red blood cells from oxidative damage. The reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), a coenzyme that keeps the intracellular pool of reduced glutathione (GSH) stable and shields cells from oxidative damage, is produced by G-6-PD. Due to their lack of nuclei and mitochondria, red blood cells are especially affected by G-6-PD deficiency. As a result, they must rely entirely on G-6-PD to generate NADPH and reduced glutathione, which shields them from oxidative damage (Nkhoma et al., 2020). Nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to NADPH when glucose-6-phosphate (G-6-P) is oxidized to 6-phosphogluconate (6-PG) by this enzyme in the hexose monophosphate shunt. The reduced form of glutathione (GSSG) is maintained by NADPH, a necessary co-factor in numerous biosynthetic pathways (GSH). To prevent oxidized haemoglobin from precipitating as Heinz bodies, reduced glutathione helps convert oxidized haemoglobin into free haemoglobin by acting as a scavenger for free radicals. Since G-6-PD activity is the only source of NADPH that shields red blood cells from oxidative stress, they are entirely dependent on it, even if many other body cells have alternative ways of producing NADPH (Obasa et al., 2021).

Among human hereditary erythrocyte enzymatic disorders, glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most prevalent and

pervasive. About 400 million people worldwide suffer from glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-linked hereditary enzymatic abnormality, with populations in Africa, South-East Asia, the Mediterranean region, and the Far East being the most affected (Beutler et al., 2020; Monchy et al., 2024). In the United States, the prevalence is 12.8%, in India it is 3.9%, and in the Middle East it is 50% (Obasa *et al.*, 2021). G6PD deficiency affects 4–26% of Nigerians, with 20–26% of men having the condition (Luzzatto and Gordon-smith, 2021; Ademowo and Falusi, 2022). Each community has a different prevalence rate. There are several variations in the gene that codes for G-6-PD; some result in minor deficits, while others produce severe deficiencies (Recols *et al.*, 2022; Zaffanello *et al.*, 2024). Because the gene is found on the X chromosome, males can only be hemizygous (have only one copy) for the gene, but females can be homozygous or heterozygous. Consequently, heterozygous women have two red blood cell populations, each of which is caused by the expression of one of two G-6-PD alleles, because of lyonization (the inactivation of one X chromosome) (LaRue et al., 2020). Numerous tests can accurately identify X-chromosomally transmitted G-6-PD deficiency in hemizygous males and homozygous women. Due to lyonization, it is challenging to diagnose G-6-PD deficiency in heterozygous women, and a significant portion of this population is overlooked. The cytochemical assay is the only accurate method to distinguish between heterozygous-deficient and non-deficient or homozygous women (Recols *et al.*, 2022; Zaffanello *et al.*, 2024). A G-6-PD deficit increases the risk of kernicterus, or serious brain damage, in the first few days of birth and leads to neonatal jaundice, which is accompanied by hyperbilirubinemia. Permanent brain damage, mental issues, and hearing impairments can result from kernicterus. Many children with G-6-PD deficiency are healthy during their childhood until they encounter a chemical or drug that contains pro-oxidants. Antimalarial medications are traditionally powerful antioxidants and are widely used in Sub-Saharan Africa. Furthermore, young children are frequently exposed to the pro-oxidant naphthalene, which is the active component of mothballs. Pro-oxidant exposure in G-6-PD deficiency can cause a quick shift in the redox state of red blood cells, which can cause haemolysis and lead to severe

anaemia, heart failure, and even death if not detected in time (Olatundun et al., 2023). The abrupt breakdown of the older, more enzyme-deficient erythrocytes cause haemolysis. According to Akanni et al. (2020), this happens after exposure to some medications with high redox potentials, mothballs, henna, Fava beans, or after specific illnesses and metabolic processes. In areas where the frequency of G-6-PD insufficiency is 3–5% or higher, the WHO advises population screening. However, in many parts of Nigeria, this has not yet become standard procedure. Cost, the medical community's underestimation of the public health impact of G-6-PD deficiency, laypeople's ignorance of the condition, and a lack of guidelines about which high-risk groups should be preferentially screened when general population screening is not feasible are some of the obstacles to screening (Olatundun et al., 2023). This review provides clear insight on the molecular mechanisms, pathophysiology, laboratory investigation and possible therapeutic approaches for Glucose-6-Phosphate dehydrogenase deficiency.

2.0 Historical review

A clinical manifestation of G-6-PD deficiency closely related to drug-induced haemolysis is the haemolytic anaemia induced by ingestion of the fava bean (*Vicia faba*). Favism has been known since antiquity. Indeed, the demise of Pythagoras has been attributed to unwillingness to enter a bean field, possibly because of favism (Arie, 1959). The earliest suspected reports of G-6-PD deficiency are from Pythagoras forbidding his students from eating fava beans, his strong aversion to these commonly eaten beans must mean that favism had already been recognised as a dangerous disease and since G-6-PD deficiency was common in Greece as at that time. It is possible that he or some of his followers may have suffered from favism, the haemolytic condition triggered by ingesting fava beans (Simoons, 1998). Fava beans are unique among other beans because they contain high concentration of two Glucosides, vicine and divicine and their irrespective aglycones convicine and isouramil which are powerful triggers of oxidative stress that causes the characteristic haemolytic attacks (Chevion *et al.*, 2019; Luzzatto and Poggi, 2020). However, because the response to fava beans ingestion is

inconsistent, popular theories on the pathogenesis of favism were related to toxic effect or allergy (Cappellini and Fiorelli, 2018).

In the year 1926, Cordes reported the occurrence of acute haemolysis in individuals treated for malaria with 6-methoxy-8-aminoquinoline drugs, however the mechanism of haemolysis could not be understood for the next three decades (Cordes, 1926). The discoveries that lead to the recognition of G-6-PD deficiency were the result of several convergent events. The biochemical pathways through which red cells metabolize sugar were established (Beutler *et al.*, 2020; Beutler, 2022). The tools available then seem incredibly primitive today, but by 1950, virtually every step in red cell glycolysis was established. Secondly, the development of isotopic methods permitting the accurate estimation of red blood cell survival was essential. It was the ⁵¹Cr method of labelling erythrocytes, first devised by Sterling and Gray to measure the red cell mass and subsequently adapted for measuring the cell survival, that set the stage for the studies that had to be performed (Sterling *et al.*, 2020). Finally, the circumstances surrounding World War II and the Korean War had created the necessity for the development of new, synthetic antimalarial drugs. The funding required to carry out the studies materialized from the United States Army. Volunteers serving sentences in the Illinois State Penitentiary at Joliet were selected for conducting the clinical investigation on understanding why 8-amino quinoline antimalarials caused haemolysis (Beutler, 2020). When normal volunteers were given 30 mg of primaquine daily, some developed acute haemolytic anaemia; most did not. Did those who develop haemolytic anaemia metabolize this 6-methoxy-8-amino quinoline antimalarials drug differently, or did their red cells differ in some manner? Development of ⁵¹Cr technique made it possible to answer this question. When ⁵¹Cr labelled cells from a primaquine-sensitive subject were transfused into a non-sensitive subject, primaquine administration resulted in rapid destruction of the labelled erythrocytes. But when ⁵¹Cr labelled cells from a non-sensitive subject were transfused into a primaquine sensitive subject, they survived normally even when the host's red cells were being rapidly destroyed. These studies established clearly that sensitivity to

the haemolytic effect of primaquine was due to an intrinsic defect of the erythrocyte (Beutler, 2020).

There were few morphological changes in the red cells of subjects undergoing primaquine-induced haemolysis, but Heinz bodies could be detected in the red blood cells in circulation before the onset of haemolysis. Heinz bodies can be induced in vitro by compounds such as phenyl hydrazine or acetyl phenyl hydrazine, and the pattern of in vitro Heinz body formation was quite different in sensitive and non-sensitive subjects. (Beutler *et al.*, 2020). This made it possible, for the first time, to detect primaquine sensitivity in vitro. The fact that iodoacetate and arsenate caused normal cells to behave like primaquine sensitive cells focused attention on the sulfhydryl groups of the erythrocyte. The amount of glutathione in the cells was diminished, and the ability of erythrocytes to maintain glutathione levels in the face of oxidative stress was abnormal (Beutler *et al.*, 2020). Later, Carson studied the reduction of glutathione by haemolysates. When it was found that haemolysates from primaquine sensitive men could not utilize glucose-6-phosphate to reduce glutathione, the more expensive NADPH was added as the hydrogen donor. This substrate could reduce glutathione in haemolysates from primaquine sensitive men, making it apparent that the primary defect was in the glucose-6-phosphate utilizing enzyme, G6PD (Carson *et al.*, 2019). Crosby also noted a similarity between the severe haemolytic anaemia associated with ingestion of fava beans or even inhalation of the plant pollen and the haemolytic anaemia induced by primaquine (Cappellini and Fiorelli, 2018). The discovery of G-6-PD deficiency is a clear example of how clinical studies carried out on prisoner volunteers can benefit society and save lives without harm to anyone (Beutler, 2021).

3.0 Epidemiology

Deficiency of glucose-6-phosphate dehydrogenase (G6PD) is one of the most common and widespread human hereditary erythrocyte enzymopathies. Glucose-6-phosphate dehydrogenase G-6-PD deficiency is an X-linked hereditary enzymatic abnormality that affects globally about 400 million people (Beutler *et al.*, 2020; Monchy *et al.*, 2024) and particularly African populations, Black

Americans, South-East Asian populations, populations around the Mediterranean and the Far East (Meletis *et al.*, 2024; Xavier, 2022). In 2010, it resulted in about 4,000 deaths globally (Lozano, 2022). In Nigeria, the prevalence of G6PD deficiency ranges from 4 – 26% with the male population having about 20 – 26% (Luzzatto and Gordon-smith, 2021; Ademowo and Falusi, 2022). This prevalence rate varies from one community to another. Specific prevalence rates worldwide range from 3.9% in India, 12.8% in the USA and 50% in the Middle East (Obasa *et al.*, 2021). The frequency of mutation varies greatly among different population (Nkhoma *et al.*, 2020). In the United States, black males are primarily affected, with a prevalence of about 10%. Internationally, the geographic prevalence of the disorder correlates with the distribution of malaria, a disease with high mortality and morbidity rate (Beutler, 2018). Hence G-6-PD deficiency is protective against malaria infection (Peter and Van Noorden, 2022). Thus, malaria parasite growth is slowest in G-6-PD deficient cells than healthy cells (Cappellini and Fiorelli, 2018).

However, because of recent migration of individuals from areas with high prevalence of G6PD deficiency, deficient alleles have been quite prevalent in North and South American and in parts of Northern Europe (Frank, 2020). Therefore, G-6-PD deficiency is prevalent in all five continents, but it has never been reported in Amerindians, and one might speculate that this is related to the fact that they have been exposed to malaria only over the past three centuries (Luzzatto and Poggi, 2020). In most of the areas with high prevalence of G-6-PD deficiency, several polymorphic alleles are found but the tropical region of Africa is one exception where the variant G-6-PDA- account for about 90% of G-6-PD deficiency and G-6-PD A- which is also frequent in North and South America, in the West India's and in areas where persons of African origin are present. Moreover, G-6-PDA- is quite prevalent in Italy, the Canary Island, Spain and Portugal and in the Middle East including Iran, Egypt and Lebanon (Cappellini and Fiorelli, 2020). The Mediterranean variant of G-6-PD deficiency is present in all countries surrounding the Mediterranean Sea (Luzzatto *et al.*, 2021). Although it is also widespread in the Middle East including Israel,

where it accounts for almost all G-6-PD deficiency in Kurdish Jews, India and Indonesia. In several populations such as the countries around the Persian Gulf, G-6-PD A- and G-6-PD Mediterranean coexist at polymorphic frequencies (Cappellini and Fiorelli, 2018). In some population of Kurdish Jews, the gene frequency exceeds 50% (Prchal and Gregg, 2023). Several variants are pandemic in Asia, G-6-PD Mahidol is common in Thailand and India, G-6-PD Chinese -1, G-6-PD Chinese-2, G-6-PD Chinese – 3 and G-6-PD Canton are equally frequent in China and Southeast Asia. Mutations that arise in parts of the world not plaque by malaria are Sporadic. The severity of G6PD deficiency varies significantly among racial groups. Variants producing severe deficiency primarily occur in the Mediterranean population. African populations have milder haemolysis due to higher enzyme levels. (Prchal and Gregg, 2023).

4.0 Genetics

G6PD enzyme is encoded by a human X-linked gene (Xq2.8) consisting of 13 exons and 12 introns, spanning nearly 20 kb in total; the first exon is noncoding, while the remaining 12 range from 120 to 235 bp (Fig.1) (Cappellini and Fiorelli, 2018; Luzzatto, 2021; Peter and Van, 2022). With the sequence of the entire gene known (Peter and Van, 2022; Singh *et al.*, 2023). The gene encodes for 515 amino acid and a Guanine-Cytidine (GC) rich (more than 70%) promoter region. All mutation of the G-6-PD gene results in enzyme deficiency affecting the coding sequence (Cappellini and Fiorelli, 2018). About 140 mutations being reported of which most are single base substitution leading to amino acid replacement (Hirono *et al.*, 2022). The inheritance of the G-6-PD deficiency shows a typical X-Linked pattern which was initially identified through Favism having a higher incidence in males than in females long before G-6-PD deficiency was identified as the cause (Cappellini and Fiorelli, 2018).

There was however an X-linkage of primaquine sensitivity long before the basic defect was well established and the X-linkage of primaquine sensitivity was established by the application of the Glutathione stability test. The earliest reliable means for the detection of the defect of primaquine sensitivity was however carried out

among the Afro-American families which showed that glutathione instability was most frequently transmitted from mother to son although there were instances in which the defect could not be detected in the mother and even where apparent father to son transmission occurred, it was correctly presumed that these anomalies were caused by inadequate ascertainment of heterozygous females with the crude technology that was in place as at that time but with the recognition of the basic defect which was a deficiency of the G-6-PD gene, X-linkage was then confirmed by the estimation of enzyme activity, studies of electrophoretic mobility and study of linkage with colour blindness. Although there were still females in which genetic transmission aberrant for X-linkage was observed and this suggested that one of the two X-chromosome might be inactive in human females (Beutler, 2018). The G-6-PD gene is designated as *Gd* (Luzzatto and Poggi, 2020).

The term G-6-PD normal and G-6-PD deficient are used to designate the phenotype of persons while G-6-PD (+) and G-6-PD (-) are used for the genotype of individual cells, because G6PD is X-linked, males can only be normal hemizygote (*Gd*⁺) or deficient hemizygote (*Gd*⁻) while females can be normal homozygote (*Gd*⁺/*Gd*⁺), deficient homozygote (*Gd*⁻/*Gd*⁻) or heterozygote (*Gd*⁺/*Gd*⁻) with the phenotype of the heterozygote female often referred to as intermediate because their overall red cell G-6-PD level usually lies in between the normal and the deficient range (Luzzatto and Poggi, 2020). Hence the heterozygote female are genetic mosaics (Cappellini and Fiorelli, 2018). Owing

to random inactivation of one out of the two X-chromosomes and this is known as Lyonization, where one erythrocyte population is G-6-PD deficient and the other population with a normal G-6-PD activity (Peter and Van, 2021). With the abnormal cells of the heterozygous female deficient for G-6-PD enzyme as those of a G-6-PD deficient male, such females can be susceptible to the same pathophysiological phenotype. Although such females on the average have less severe clinical manifestation than G-6-PD deficient males, however in some population with high frequency of G-6-PD deficient allele, homozygous females are not rare (Cappellini and Fiorelli, 2018).

The X-linkage of *Gd* has three major consequences;

- i. *Gd* mutation displays the typical pattern of mendelian X-linked inheritance.
- ii. Severe G-6-PD deficiency is much more common in males than in females.
- iii. As a result of X-chromosomes inactivation, female heterozygous for two different *Gd* alleles exhibit somatic cell mosaicism (Luzzatto and Poggi, 2020).

The *Gd* is in a cluster of genes on the distal long arm of the X-chromosome (locus q28) (Beutler, 2008; Betler *et al.*, 2018 and Peter and Van, 2021). The genes for Fragile x, haemophilia A, colour blindness, a putative gene for bipolar affective illness, the ABP-280 Filamin (FLN), Bornholm eye disease, Clapsed-thumb mental retardation (MASA) syndrome and Dyskeratosis congenital are also located here (Beutler, 2018).

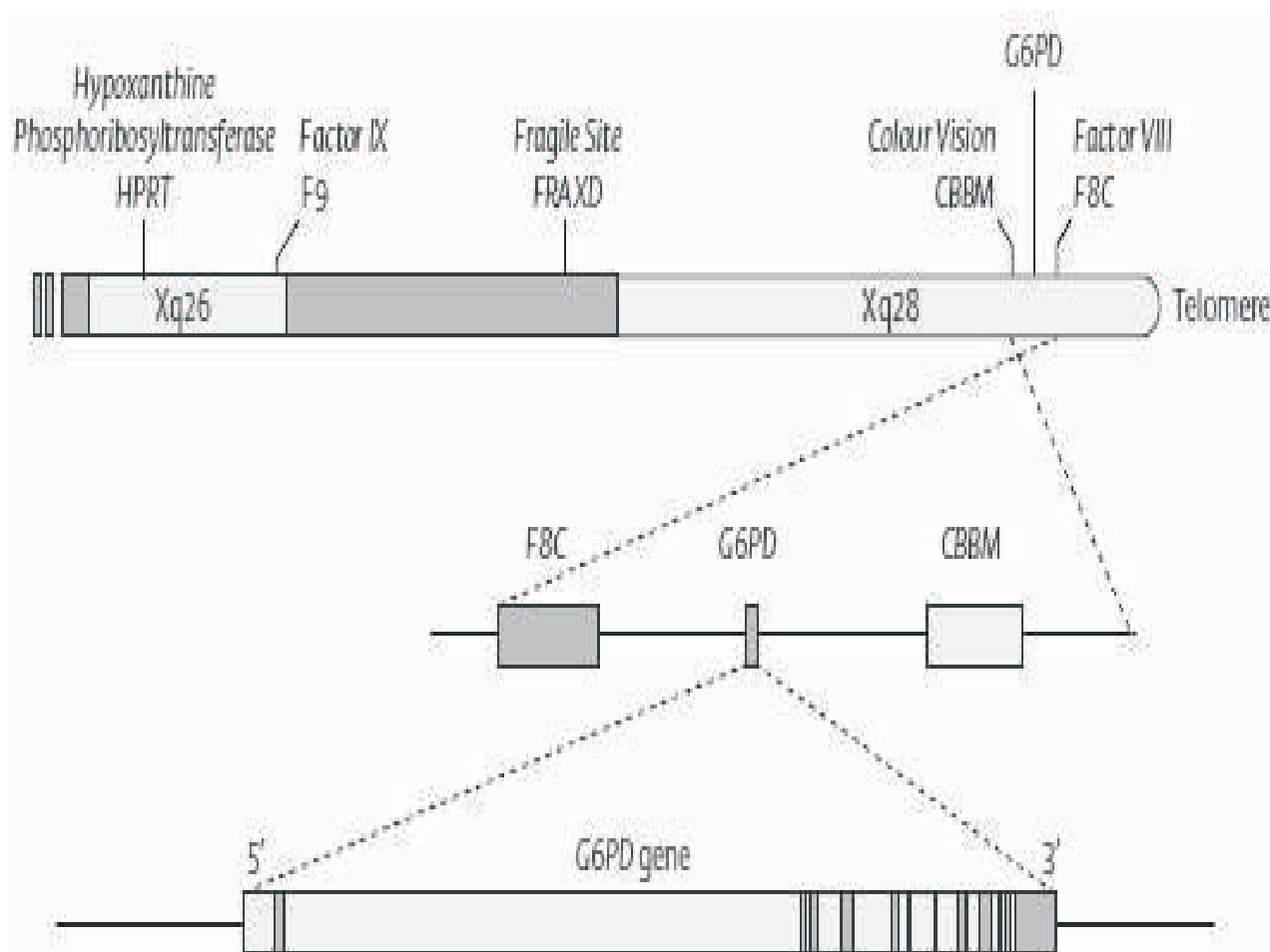


Figure 1: Location of G-6-PD Gene on the X-Chromosome (Cappellini and Fiorelli, 2018).

5.0 G-6-PD and G-6-PD Enzyme

The monomer of human G6PD consists of 514 amino acids with a calculated molecular weight of 59 kDa (Persico *et al.*, 2020). The presence of tightly bound NADP⁺ in the human erythrocyte enzyme and the effects of oxidised and reduced coenzyme and pH on stability and oligomerisation have been reported over many years (Cohen and Rosemeyer, 2020; Scopes *et al.*, 2022). The active human enzyme exists in a dimer-tetramer equilibrium. High pH and ionic strength shift the equilibrium towards the dimer, whereas low pH conditions cause a shift towards the tetramer (Cohen and Rosemeyer, 2021) inactive monomers might also be formed at high pH. Electron micrographs show that tetramers pre-dominant below pH 6 and dimers predominate above pH 8 (Wrigley *et al.*, 2020). In addition, EDTA, NADPH and G6P each favour disruption of the dimer, whereas NADP⁺ or certain metal ions favour the tetramer (Krikman *et al.*, 2019; Scopes *et al.*, 2022). Little

is known about the physiological significance of the interconversion.

Analysis of the three-dimensional model of human G-6-PD enzyme, obtained from the crystallised protein, has indicated that the NADP⁺ binding site is in a part of the enzyme close to the N-terminus, with the highly conserved amino acid (in 23 species) Arg 72 playing a direct part in coenzyme binding (Peters and Van Noorden, 2022). With a pH dependent equilibrium (Cappellini and Fiorelli, 2018). Each subunit binds to the NADP⁺ molecule for its structural stability which is positioned close to the interface where the two subunits of each dimer bind (Au *et al.*, 2020). The dimer structure of the two subunits in the enzyme are symmetrically located across a complex interface of β -sheets (Au *et al.*, 2020). Thus NADP⁺ appears to be bound to the enzyme both as a structural component and as one of the substrates of the reaction (Beutler, 2018). Most

of the mutation disrupt the enzyme structural stability and thus reduce its overall activity (Mehta *et al.*, 2020). Hence most mutations are point mutations and small deletions that cause structural defects in the enzyme. One mutation causes a frame shift. The lack of severe mutations indicates that total G-6-PD deficiency is lethal. In most cases, mutations cause instability of the enzyme or altered activity, usually by decreased affinity of G-6-PD for its substrates, NADP or glucose-6-phosphate (G-6-P) (Luzzatto 2021). The effect of each mutation on enzyme structure and function depends on the location of the substituted amino acid (Mehta *et al.*, 2020), hence the mutations that cause the most severe disease phenotype, hereditary non-spherocytic haemolytic anaemia tend to occur in the regions of the subunit near the tightly bound “structural” NADP (Beutler, 2018). As with all enzymes, G-6-PD activity decreases with cell age, it is estimated that in normal red blood cell, reticulocytes have about five times higher activity level than the oldest 10% of red blood cells (Luzzatto and Poggi, 2020; Luzzatto, 2021). Erythrocytes are not able to synthesize new proteins and compensate for poor G-6-PD function. As the deficient erythrocyte ages, the quantity of active G-6-PD diminishes rapidly, increasing the vulnerability of not only old but also younger erythrocytes to oxidative stress (Peters and Van Noorden, 2022).

5.1 Function of G6PD Enzyme

The role of G6PD in maintaining the integrity of red blood cell membrane cannot be overemphasized. G6PD is an essential enzyme whose major function is to keep the haemoglobin iron in the reduced physiologically active ferrous state for oxygen transport and to protect haemoglobin from oxidative damage. It catalyses the first step in the hexose monophosphate pathway, producing nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH) (Luzzatto and Gordon-smith, 2021). It catalyses the oxidation of Glucose-6-Phosphate (G6P) to 6-phosphogluconate (6-PG) and concomitantly, Nicotinamide Adenine Dinucleotide Phosphate (NADP⁺) is reduced to NADPH. The NADPH is a required co-factor in many biosynthetic reactions, maintains Glutathione (GSSG) in its

reduced form (GSH). Reduced Glutathione acts as a scavenger for free radicals and thus helps reduce oxidized haemoglobin to free haemoglobin, otherwise oxidized haemoglobin will precipitate as Heinz bodies. While many other body cells have other mechanism of generating NADPH, the red blood cells rely completely on G-6-PD activity because it is the only source of NADPH that protects the red cell against oxidative stress (Obasa *et al.*, 2021).

5.2 G-6-PD Deficient Variants

According to WHO criteria (Betke *et al.*, 2018), so far over 442 different variants of G-6-PD have been identified by the standardized enzymological methods and grouped in five classes on the basis of their activity and clinical manifestations (Betke *et al.*, 2018). G-6-PD deficiency usually becomes manifest in most cases in hemizygous males (Betke *et al.*, 2018). The clinical presentations of the G-6-PD - deficient subjects of Asian origin appear to be different from those of African Americans with G-6-PD deficiency (Beutler, 2020). This may be due in part to the fact that most African Americans with G-6-PD deficiency are asymptomatic (Beutler, 2020). Approximately 20% to 40% of neonatal jaundice is related to G-6-PD deficiency in Taiwan. In contrast, neonatal jaundice has rarely been attributed to G-6-PD deficiency in the United States (Hirono *et al.*, 2022). In 1967, the World Health Organisation made initial recommendation for the characterisation of G-6-PD deficiency, and these have subsequently been updated (Betke *et al.*, 2018, WHO, 2020). The method used for characterizing the enzyme variants are:

- Red cell G-6-PD activity
- Electrophoretic migration
- Michaelis constant (K_m) for Glucose-6-Phosphate (G6P)
- Relative rate of utilization of 2-deoxyglucose-6-Phosphate (2dG6P)
- Thermal stability

Other desirable's studies may include the determination of P^H optima, the K_m of NADP, the heat of activation, the response to inhibitors and the migration on chromatographic media (Betke *et al.*, 2018).

Examination of G-6-PD variants shows that in most cases, G-6-PD deficiency is due to enzyme instability, implying that amino acid substitutions in different location can destabilise the enzyme molecule hence the G-6-PD enzyme deficit can be caused by a reduction in the number of enzyme molecules, a structural difference in the enzyme causing a qualitative change (Cappellini and Fiorelli, 2018). The differences between some variants are subtle and most likely reflect minor technical differences between laboratories rather than true enzyme defects. Moreover, the advances in molecular biology have revealed that many so-called G-6-PD variants have the same DNA defect. The normal wild-type enzyme defect, G-6-PD B⁺ found in most whites and Asians and in most of the Blacks' population, has a normal catalytic activity and is not associated with haemolysis (Glader, 2019). G-6-PD B⁺ is the most commonly found enzyme variant type and is used as a standard for normal enzyme activity, electrophoretic mobility and for identification of other variants. The rate at which NADP⁺ is reduced by glucose-6-phosphate with G-6-PD B⁺ as the catalyst is the standard for activity. Based on this, enzyme activity relative to G-6-PD B⁺ variants are classified as fast, normal, and slow in terms of electrophoretic mobility and as Classes I—V (Betke *et al.*, 2018; Beutler, 2020; Greene, 2021 and Segel, 2022). However, when the nucleotide structure of the wild-type enzyme G-6-PD B⁺ was established, some of these previously described different variants were found to represent identical mutations although in some instances they arose independently on different haplotypes of the G-6-PD gene (Prchal and Gregg, 2023). Most of the mutations are missense. Mutations associated with chronic haemolysis tend to cluster in the vicinity of the

NADP binding domain of the G-6-PD gene while those associated with acute haemolysis or no haemolysis are scattered throughout the gene. Unlike disease causing mutations of other genes, deletion and insertion causing frame shift and stop codon mutations are not observed, these events would be expected to be fatal since G-6-PD is a housekeeping gene essential for basic cellular functions. All the G-6-PD deficient variants that occur at a high frequency are African G-6-PD A⁺ variant and the Mediterranean variant, several variants are also present in Southeast Asia. All these variants cause acute intermittent haemolysis (Prchal and Gregg, 2023). In the African (G-6-PD A⁺) variant and Mediterranean variants, the abnormal enzyme may be synthesized at normal or near normal rates but has decreased stability *in vivo*. The amount of enzyme antigen in the red cells declines concurrently with enzyme activity. This suggests that the mutant protein in these variants is rendered unusually sensitive to proteolysis in the environment of the erythrocyte (Beutler, 2018).

5.1.1 African Variant

Among persons of African descent, a mutant enzyme with normal activity is very prevalent designated as G-6-PD A⁺ (Beutler, 2018). It is found in 20 – 40% of African men and 20% of African American men (Beutler, 2018). It has normal catalytic properties and does not cause haemolysis, it differs from normal G-6-PD B⁺ enzyme in that it has a faster electrophoretic mobility (Glader, 2019). This variant has 90% of the enzyme activity of G-6-PD B⁺ (Luzzatto, 2021). The structure of G-6-PD A⁺ differs from that of G-6-PD B⁺ by the substitution of a single amino acid, an Asparagine for Aspartate at the 126th position (126Asn

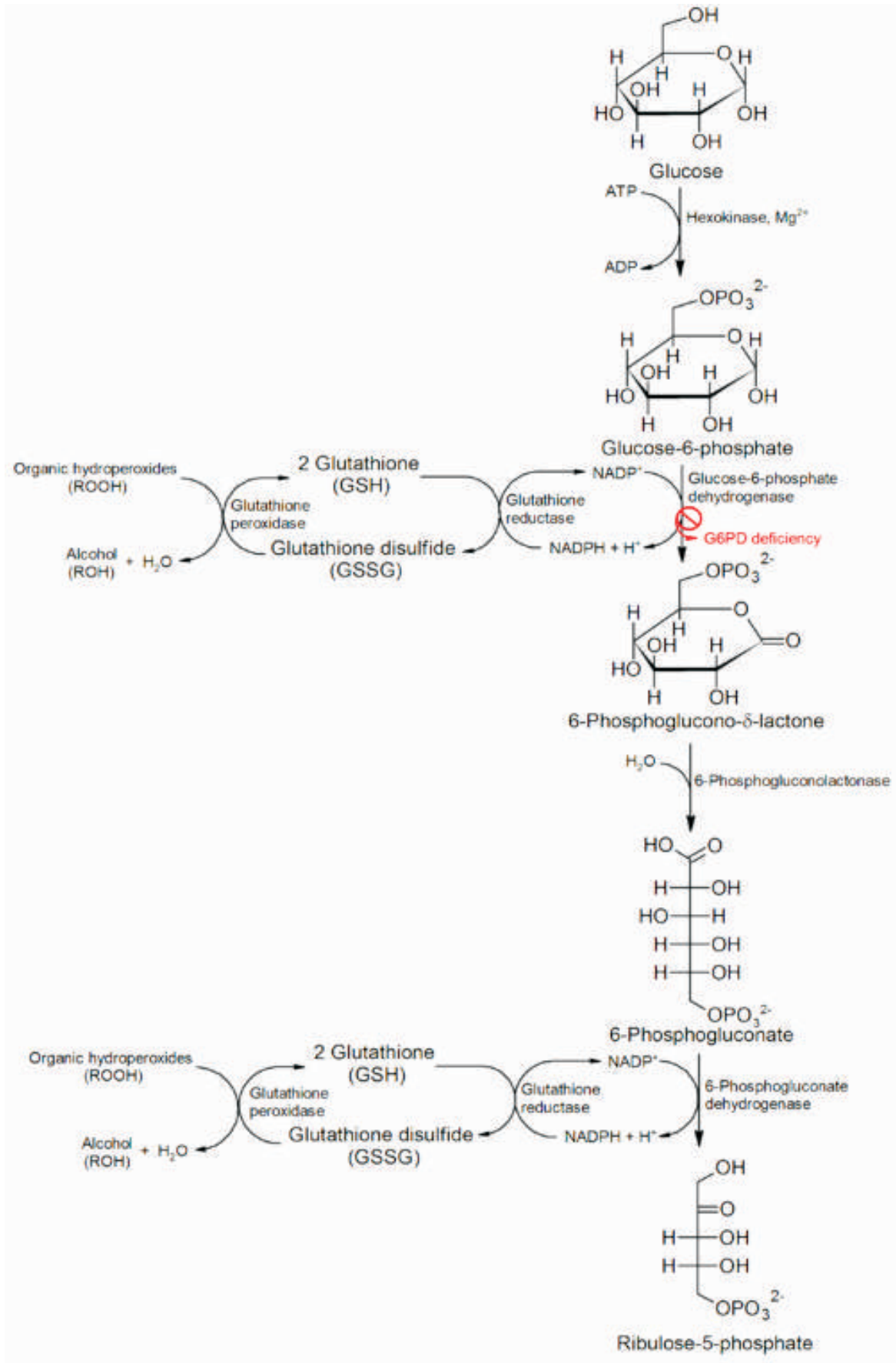


Figure 2: The Pathway of Haemolysis (Gaskin *et al.*, 2021).

7.0 Clinical Manifestation

The clinical spectrum of G6PD deficiency has been identified in a vast number of conditions. Clinical manifestations depend on the degree of the enzyme deficiency, which in turn is determined by the characteristics of the G6PD variant. Majority of individuals are asymptomatic and do not have haemolysis in the steady state. They have neither anaemia, evidence of increased red blood cell destruction, nor an alteration in blood morphology, although a modest shortening of RBC survival can be demonstrated by isotopic techniques. However, episodes of acute haemolysis with haemolytic anaemia may be triggered by medications, certain foods, and acute illnesses, especially infection (WHO, 2020). The clinical manifestation will be discussed under the following heading;

- Drug induced haemolytic anaemia
- Infection induced haemolytic anaemia
- Food induced haemolytic anaemia
- Diabetic ketoacidosis
- Neonatal jaundice
- Congenital non-spherocytic haemolytic anaemia.

7.1 Drug Induced Haemolysis

The haemolytic effect of primaquine in G6PD deficient individuals were already established. But later, it was realized that the spectrum of drugs that caused haemolysis was much more than that had been anticipated. Transfusing labelled red cells from a few primaquine sensitive donors into a larger number of non-sensitive recipients and then challenging the sensitive cells with a variety of drugs made it possible to quantitate the extent to which each drug produced haemolysis (Dern *et al.*, 2018; Brewer *et al.*, 2019; Beutler, 2020). Table 2.1 lists drugs that have been shown to be capable of producing clinically significant haemolytic anaemia in doses that are normally used. Table 2.2 indicates which drugs can be given safely to most patients with G6PD deficiency (Beutler, 2020; Beutler *et al.*, 2020).

7.2 Foods Induced Haemolysis

Certain foods can induce haemolysis in a G6PD deficient individual. Intravascular haemolysis induced by ingestion of fava beans, known to as favism, is a well-known cause and is seen predominantly in young males age 1 to 5 years.

Symptoms include headache, back pain, chills, and fever within 24 hours of ingestion, followed by haemoglobinuria and jaundice. An important thing to be noted is that all patients with favism are G6PD deficient, but many G6PD deficient individuals can eat fava beans with impunity. Thus, the deficiency is a necessary but not sufficient cause of haemolysis. It has been suggested that the glycosides divicine and isouramil are the components of the bean responsible for a haemolysis, but there is no rigorous proof that this is the case, and the additional factor that makes a minority of G6PD deficient individuals develop haemolysis when they ingest the bean is still unknown, although it is probably inherited (Luzzatto and Poggi, 2020). Prevention campaign in areas with high prevalence of G-6-PD deficiency through neonatal screening and Health Education has greatly reduce the incidence of favism (Mehta, 1994 and Cappellini and Fiorelli, 2018).

7.3 Infection

Infection is the most common cause of acute haemolysis in G6PD-deficient persons, although the exact mechanism by which this occurs is unknown. Leukocytes may release oxidants during phagocytosis that cause oxidative stress to the erythrocytes; however, this explanation alone would not account for the variety of infections associated with haemolysis in G6PD deficient persons. The most common infections agents causing haemolysis include *Salmonella*, *Escherichia coli*, beta-haemolytic *streptococci*, rickettsial infections, viral hepatitis, and influenza A (Beutler, 2018).

7.4 Neonatal Jaundice

Anaemia and jaundice are often first noted in neonates with severe G-6-PD deficiency. The principal cause of neonatal icterus in G-6-PD deficient infants is the inability of the liver to adequately conjugate bilirubin. This problem is compounded when the infant also inherits the Uridine Diphosphoglucuronosyl Transferase-1 (UDPGT-1) promoter polymorphism that is associated with Gilbert disease. As noted, hyperbilirubinemia is likely secondary to impairment of bilirubin conjugation and clearance by the liver leading to indirect hyperbilirubinemia. G-6-PD deficiency can lead to an increased risk and earlier onset of hyperbilirubinemia, which may require phototherapy or exchange transfusion. In certain populations, hyperbilirubinemia secondary to G-6-

PD deficiency results in an increased rate of kernicterus and death, whereas in other populations this has not been observed. This may reflect genetic mutations specific to different ethnic groups. Most common variants have been associated with the syndrome including the A- and Mediterranean variants. In parts of the world where the mutations are common, the deficiency is the most prevalent cause of neonatal jaundice (Edward, 2020). Jaundice is usually evident by 1-4 days of age like physiological jaundice but is seen later than in blood group alloimmunisation (i.e., Rhesus incompatibility) (Cappellini and Fiorelli, 2018). Although there are two major differences between jaundice due to incompatibility of blood group and jaundice due to G-6-PD deficiency.

1. The presence of jaundice in G-6-PD deficiency is very rare during childbirth and usually begins in the second or third day of life
2. According to anaemia, jaundice is more pronounced, and it is encountered with severe anaemia very rarely in the absence of the enzyme (Luzzatto *et al.*, 2021).

G-6-PD deficiency and neonatal jaundice vary widely in their frequency and severity in different populations (Cappellini and Fiorelli, 2018) although genetics, cultural and environmental factors such as maternal exposure to oxidant drugs, herbal remedies or the effect

of Naphthalene-camphor balls that are sometimes used to preserve babies cloths, prematurity, breast feeding, inhalation, acidosis, hypoxia, infection such as viral hepatitis and ingestion of fava beans (Favism in utero) by the mother before delivery, are some of the factors that can exacerbate neonatal jaundice in some cases by causing haemolysis in G-6-PD deficient infants as well as the difference in their frequency and severity among populations (Cappellini and Fiorelli, 2018; Glader, 2019; WHO working Group, 2020; Mehta, 2020; Luzzatto and Poggi, 2020). Neonatal jaundice is more typical and more severe in premature infants with G-6-PD deficiency than in babies within the normal gestation period (Cappellini and Fiorelli, 2018). In summary, there are two different types of neonatal jaundice associated with G-6-PD deficiency.

- A more common type which can be visualized as a marked exaggeration of “physiologic jaundice”, this type is usually clinical benign and not greatly influenced by the environment.
- A rarer, frankly haemolytic type, more severe and can be visualized as acute haemolytic anaemia occurring in new-born baby because the baby happened to be exposed to one of the same agents that could cause acute haemolytic anaemia even in adult (Luzzatto and Poggi, 2020).

Table 2.1: Drugs and Chemicals to Be Avoided by Persons with G-6-PD Deficiency

-
- Acetanilid
 - Diaminodiphenylsulfone
 - Furazolidone (Furoxone)
 - Glibenclamide
 - Henna (Lawsone)
 - Isobutyl nitrite
 - Methylene Blue
 - Naphthalene
 - Niridazole (Ambilhar)
 - Nitrofurantoin (Furadantin)
 - Phenazopyridine (Pyridium)
 - Phenylhydrazine
 - Primaquine
 - Sulfacetamide
 - Sulfanilamide
 - Sulfapyridine
 - Thiazolesulfone
 - Trinitrotoluene (TNT)
 - Urate oxidase
 - Acetylsalicylic acid (aspirin)
 - Antipyrine
 - Chloroquine
 - L-Dopa
 - Vitamin K (Glader, 2019).
-

Table 2.2 Drugs that probably can be Safely given in Normal Therapeutic Doses to G-6-PD-Deficient Patients without Non-Spherocytic Haemolytic Anaemia

-
- Acetaminophen (paracetamol, Tylenol, Tralgon, hydroxyacetanilide)
 - Acetophenetidin (phenacetin)
 - Aminopyrine (Pyramidon, aminopyrine)
 - Antazoline (Antistine)
 - Ascorbic acid (vitamin C)
 - Benzhexol (Artane)
 - Chloramphenicol
 - Chlorguanidine (Proguanil, Paludrine)
 - Colchicine
 - Diphenhydramine (Benadryl)
 - Isoniazid
 - Menadione sodium bisulfite (Hykinone)
 - p-Aminobenzoic acid
 - p-Aminosalicylic acid
 - Phenylbutazone
 - Phenytoin
 - Probenecid (Benemid)
 - Procainamide hydrochloride (Pronestyl)
 - Pyrimethamine (Daraprim)
 - Quinine
 - Streptomycin
 - Sulfacytine
 - Sulfadiazine
 - Sulfaguanidine
 - Sulfamerazine
 - Sulfamethoxazole (Gantanol)
 - Sulfamethoxypyridazine (Kynex)
 - Sulfisoxazole (Gantrisin)
 - Tiaprofenic acid
 - Trimethoprim
 - Tripelethamine (Pyribenzamine) (Glader, 2019)
-

7.5 Congenital Non-Spherocytic Haemolytic Anaemia and Chronic Haemolysis

Chronic haemolysis is not characteristic of most individuals with G6PD deficiency, but those with severe deficiency can have chronic haemolysis with or without chronic anaemia. Variants that produce chronic haemolytic anaemia are referred to as class I variants. These individuals have such severe G6PD deficiency that they may have haemolysis even in the absence of oxidant injury from medications or illnesses (Grossman and Ramanathan, 2016; Beutler *et al.*, 2020). These individuals may also be referred to as having congenital non

spherocytic haemolytic anaemia (CNSHA). The term non spherocytic is somewhat of a misnomer, since these individuals may have spherocytes on the peripheral blood smear. However, this term is useful in distinguishing individuals with G6PD deficiency, in whom spherocytes are relatively infrequent at baseline, from those with hereditary spherocytosis, in whom spherocytes are abundant. The severity of haemolysis varies, causing mild haemolysis to transfusion dependent anaemia. Exposure to oxidative stress can cause acute haemolysis in these persons (Manson, 2016).

7.6 Cardiovascular disease

The clinical effects of G6PD deficiency on the heart remains largely unexplored despite it being the most known enzyme deficiency in the world. Although studies suggest that G6PD deficiency may decrease superoxide production in failing myocardium and that G6PD deficiency may decrease the risk of developing coronary heart disease, recent studies in mice indicate increased oxidative stress in G6PD deficient failing myocardium and that G6PD deficiency adversely affects the development of heart failure (Hecker *et al.*, 2023).

The effects of G6PD deficiency on the development and progression of heart failure in human patients could be explored by screening hypertensive patients for G6PD deficiency and then following these patients over an extended period to see whether G6PD deficiency affects the development of heart failure in these patients. Another study could examine G6PD deficient patients who have already developed heart failure to determine whether G6PD deficiency positively or negatively affects prognosis. Thus, the development of heart failure should be examined in G6PD deficient patients. Overall, G6PD deficiency may decrease the rate of cardiovascular disease development among humans through its effect on atherogenesis (Hecker *et al.*, 2023). Meloni has concluded that subjects with G6PD deficient phenotype are less prone to coronary heart disease (CHD). They suggest that such a protective effect may be ascribable to a reduced 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR) activity, a statin-like effect, as well as to a down regulation in NADPH oxidase activity with a consequent reduction in oxygen free radical production (Meloni *et al.*, 2018).

7.7 G-6-PD activity in Diabetic patients

Diabetes mellitus is a common and complicated disease. Activities of enzyme G6PD is important in preventing its complications. Unsuitable control of blood glucose decreases G6PD activity and increases diabetes mellitus complications. This issue itself aggravates diabetic injury due to inappropriate antioxidation process. Simultaneous dyslipidaemia and obesity may intensify the effect of hyperglycaemia and oxidative stress. G6PD

activity level can reflect the glycaemic control and even predict subsequent complications while no present Literature has shown that G6PD mean activity in diabetics is significantly lower than non-diabetics. Various studies have concluded that reduced activity of G6PD is a risk factor for DM. Since diabetic patients simultaneously suffer from other endocrine problems like dyslipidaemia, it is worthy to evaluate the impact of such associated abnormalities on G6PD activity. Comparing G6PD activity between dyslipidaemia and non-dyslipidaemia patients within diabetics and comparing G6PD activity between non-diabetic and dyslipidaemic diabetic patients, the researchers showed that its activity was significantly lower in dyslipidaemic patients (Rashidi *et al.*, 2023). On the other hand, studies have also concluded that G6PD deficiency showed a trend for protection against diabetes with proliferative diabetic retinopathy. Further experimental and clinical studies are necessary for a better understanding of the mechanism by which G6PD deficiency may affect diabetes and its retinal vascular complications (Pinna *et al.*, 2023). Diabetic ketoacidosis has usually been considered a cause of haemolysis in G-6-PD deficient individuals but a review of 36 episodes of diabetic ketoacidosis in G-6-PD deficient subject yielded only 10 in whom haemolysis occurred and all these were associated with infection or drug ingestion. It has been suggested that hypoglycaemia may precipitate haemolysis (Beutler, 2018).

8.0 G-6-PD Deficiency and Malaria

It has been suggested that high frequency of G-6-PD deficiency in certain parts of the world is probably related to the protection it affords against *Plasmodium malariae*. This protection is largely limited to the female heterozygotes. African heterozygous females have two populations of red cells G-6-PD B (Normal) and G-6-PD A- (Deficient) due to the random inactivation of chromosomes during embryogenesis. Growth of the parasite is inhibited in the G-6-PD deficient cells. However, it has been shown that the malaria parasite can adapt to this deficiency by synthesising its own G-6-PD enzyme after 4-5 cycles in the G-6-PD deficient red cells. Therefore, parasite can grow and develop in hemizygous males (in whom all red cells are G-6-PD deficient) after a few cycles.

In female heterozygotes, the parasite may invade either G-6-PD Normal or Deficient red cells during successive cycles. Therefore, stimulus to the parasite to adapt by synthesising its own G-6-PD is considerably diminished. This results in decreased parasitaemia in female heterozygotes and protection against severe disease (Shirish, 2018). Three possible mechanisms explain the decreased intra erythrocytic parasitisation of G-6-PD deficient erythrocyte:

- The parasite is unable to invade the cells (Failed Infection)
- The intracellular environment with decreased levels of GSH and lowered activity of the hexose monophosphate shunt, prevent reproduction of the parasite (Abortive Infection)
- The parasitized red cell is destroyed by reticuloendothelial phagocytes (Suicidal Infection) (Miller, 2019).

9.0 Laboratory Diagnosis of G-6-PD

The diagnosis of G-6-PD is made when patient develops anaemia, jaundice and symptoms of haemolysis after being exposed to certain external stimuli or chemicals (in foods, drugs, mothballs) especially when there is a long family history concerning the effect of such substance. Generally, the investigation will include:

- Complete blood count
- Reticulocyte count: in active G-6-PD deficiency, Heinz bodies can be seen in red blood cell on a blood film stained supravivally.
- Liver enzyme (to exclude other causes of jaundice)
- Lactate dehydrogenase (elevated in haemolysis and a marker of haemolytic severity)
- Haptoglobin (decreased in haemolysis)
- A "direct antiglobulin test" (Coombs' test)-This should be negative, as haemolysis in G-6-PD is not immune mediated (Peter and Van, 2021).

When there are sufficient grounds to suspect G-6-PD, other tests should be carried out.

Long before it was discovered that G-6-PD deficiency is the leading cause of haemolytic anaemia, two methods for detecting individuals sensitive to the haemolytic effect of primaquine had been developed, the Heinz body test and the GSH stability test, though these tests are now

obsolete and no longer have a role in the diagnosis of G-6-PD deficiency. Instead, a quantitative assays or screening tests that detect severe deficiency are now used to diagnose the disorder (Beutler, 2018) but only a few tests can be used to diagnose G-6-PD deficiency in heterozygous female. The DNA test can be used to diagnose Hemizygous male, Homozygous female and a Heterozygous female in which primer are used to check whether the G-6-PD gene contains a mutation, but this DNA test has the disadvantages in that only one mutation can be analysed by one primer and at present about 140 mutations are known and their DNA sequence have to be known before Primer can be designed. Moreover, DNA tests are expensive and require sophisticated equipment, which makes them unsuitable for diagnosis on a large scale and impossible to apply in developing countries. Tests based on the measurement of the NADPH production capacity of G-6-PD can be used for the diagnosis of all mutations. The most frequently used tests that measure NADPH production are the fluorescent spot test, the spectrophotometric assay, and the cytochemical assay (Peter and Van, 2021).

9.1 Fluorescent Screening Test for G-6-PD Deficiency

When erythrocytes contain enough functional G-6-PD, NADP is converted into NADPH. After addition of G6P and NADP, the spot of blood fluoresces when excited at 340 nm. The fluorescent spot test is reliable for the detection of hemizygous males and homozygous females but is unreliable for the detection of heterozygous females. A sensitivity of 32% and specificity of 99% has been found for the detection of heterozygous-deficient women. The population of healthy erythrocytes in heterozygous females (on average 50% of the erythrocytes) converts enough NADP into NADPH to produce fluorescent spots, and then the heterozygous deficiency is missed. The fluorescent spot test requires a black light (a lamp that emits light of 340 nm only) (Peter and Van, 2021). The method of fluorescent screening test for G-6-PD deficiency is that of Beutler and Mitchel (Dacie and Lewis, 2016).

Principle

NADPH, generated by G-6-PD present in a lysate of blood cells, fluoresces under long-wave ultraviolet (UV) light. In G-6-PD deficiency,

there is an inability to produce sufficient NADPH; this results in a lack of fluorescence.

Method

Thaw out sufficient tubes to set up test and controls. Allow reagents to reach room temperature before use. Add 10 μ l of whole blood, either anticoagulated (EDTA, heparin, ACD [acid-citrate-dextrose], or CPD [citrate-phosphate-dextrose]) or added before clotting, to 100 μ l volumes of the reagent mixture and keep at room temperature (15-25°C). Apply 10 μ l of the reaction mixture on to a Whatman No. 1 filter paper at the beginning of the reaction and again after 5-10 min. A shorter interval may be appropriate at a high ambient temperature (25-30°C). Allow to air dry thoroughly before examining the spots under UV light. Record whether fluorescence is present (+) or absent (-). Always set up samples of normal blood and known G-6-PD-deficient blood in parallel. If the samples are to be collected away from the laboratory, place about 10 μ l of blood on Whatman No. 1 filter paper and allow it to dry. Cut out the dried blood disc in the laboratory and add it to the reaction mixture. A sample of normal blood should always be tested as a positive fluorescence (i.e., normal) control. The test can be carried out on blood stored in ACD (provided it is sterile) for up to 21 days at 4°C and for about 5 days at room temperature (Beutler and Mitchel, 2021).

Interpretation

Fluorescence is produced by NADPH formed from NADP⁺ in the presence of G-6-PD. Some of the NADPH produced is oxidized by GSSG, but this reaction, catalyzed by glutathione reductase, is normally slower than the rate of NADPH production. Red cells with less than 20% of normal G-6-PD activity do not cause fluorescence.

Like all screening tests, this method is useful when large numbers of samples are to be tested, but the result must be interpreted with caution in an individual patient. The main causes of erroneous interpretations are as follows:

1. *False-positive*-If there is reticulocytosis, a vivid fluorescence may be seen with a genetically G-6-PD-deficient blood sample because young red cells have more G-6-PD activity. If the test is carried out during an

acute haemolytic episode, the patient's blood should be retested when the reticulocyte count has returned to normal.

2. *False-negative*- If the patient is anaemic, very little fluorescence may be seen despite the G-6-PD being genetically normal, simply because there are relatively few red cells in the 10 μ l of blood used.

The test is meant to give only a positive or negative (normal or deficient) result by comparison with the controls and it does not make sense to grade by eye the intensity of fluorescence. If a control G-6-PD-deficient sample is not available, the appearance of the "zero time" spot can be used for reference. The threshold for a "deficient" result can be worked out by making dilutions of a normal blood sample in saline and is best set by regarding as deficient the fluorescence obtained when G-6-PD activity is 20% of normal or less (corresponding to a 1 in 5 dilution of normal blood). This means that very mildly deficient variants, and a substantial proportion of heterozygotes, will be missed. However, clinically important haemolysis is unlikely to occur in subjects who have more than 20% G-6-PD activity, and therefore this seems an appropriate (although arbitrary) threshold for a diagnostic laboratory. Because the test depends on visual inspection, it is best to select the time of incubation in relation to ambient temperature in preliminary trials. NADPH production is a cumulative process. Therefore, given enough time, a G-6-PD-deficient sample will fluoresce. The time allowed for the reaction should be one at which the contrast in fluorescence between a G-6-PD-normal and a G-6-PD-deficient sample is maximal (Beutler and Mitchel, 2021).

9.2 Spectrophotometric Assay

This is an enzyme assay (definitive test), it measures spectrophotometrically the rate of reduction of NADP to NADPH. An erythrocyte haemolysate is incubated with G6P and NADP, and the rate of reduction of NADP to NADPH is measured at 340nm in a spectrophotometer. This test is also reliable for the detection of hemizygous males and homozygous females. The detection of heterozygous females raises problems, because the population of healthy

erythrocytes in these women produces NADPH in a way similar to that seen in the fluorescent spot test. The sensitivity of this test for the detection of heterozygous women is 11%, whereas the specificity is 99%. The spectrophotometric assay requires a spectrophotometer (Peter and Van, 2021).

9.3 Cytochemical Tests for Demonstrating Defects of Red Cell Metabolism

Cytochemical methods have been developed by means of which some of these defects are demonstrable in individual cells. Thus, tests have been described for demonstrating red cells deficient in G-6-PD (Dacie and Lewis, 2018). The principle on which the methods are based is that red cells are treated with sodium nitrite to convert their oxyhaemoglobin (HbO_2) to methaemoglobin (Hi). In the presence of G-6-PD, Hi reconverts to HbO_2 , but in G-6-PD deficiency, Hi persists. The blood is then incubated with a soluble tetrazolium compound, which will be reduced by HbO_2 (but not by Hi) to an insoluble formazan form. Attempts have been made to improve the reliability of the test for detecting heterozygotes [e.g., by controlling slight fixation of the red cells and accelerating the reaction with an exogenous electron carrier (1-methoxyphenazine metho-sulphate)] (Dacie and Lewis, 2018) these cytochemical procedures are not more sensitive in the demonstration of G-6-PD deficiency than are the simple screening tests described above. They may, however, be useful in genetic studies and when assessing G-6-PD activity in women (Dacie and Lewis, 2018); they may be the only way to detect deficiency in the heterozygous state.

Method

Venous blood collected into ACD should be used. The test should be carried out within 8 hours of collection, and the blood should be kept at 4°C until it is tested. Centrifuge the blood at 4°C for 20 min at 1200-1500 g. Discard the supernatant and add 0.5 ml of the packed red cells to 9 ml of 9 g/l NaCl and 0.5 ml of sodium nitrite solution contained in a 15 ml glass centrifuge tube. Incubate at 37°C for 20 min. Centrifuge at 4°C for 15 min at approximately 500 g and then discard the supernatant fluid without disturbing the buffy coat and uppermost

layer of red cells. Wash the cells three times in cold saline. After the last washing, remove the buffy coat, mix the packed cells well, and transfer 50 ml to a glass tube containing 1 ml of the incubation medium. Incubate the suspension undisturbed at 37°C for 30 min. Then add 0.2 ml of MTT solution, shake gently, and incubate at 37°C for 1 hour. Resuspend the cells thoroughly. Place one drop adjacent to one drop of hypotonic saline on a glass slide, mix the drops thoroughly, and cover with a cover glass. Examine the red cells with an oil-immersion objective, noting the presence of formazan granules

Interpretation

When G-6-PD activity is normal, all the red cells are stained. In G-6-PD hemizygotes, the majority of the red cells are unstained. In heterozygotes, mosaicism is usually seen; usually 40-60% of the cells are unstained, so the proportion may be much less, and in extreme cases only as few as 2-3% may be unstained (Dacie and Lewis, 2018).

9.4 Methaemoglobin Reduction Test

Principle

Sodium nitrite converts haemoglobin (Hb) to methaemoglobin (Hi). When no methylene blue is added, methaemoglobin persists, but incubation of the samples with methylene blue allows stimulation of the pentose phosphate pathway in subjects with normal G-6-PD levels. The Hi is reduced during the incubation period. In G-6-PD-deficient subjects, the block in the pentose phosphate pathway prevents this reduction.

Method

Use anti-coagulated blood (EDTA or ACD) and test the samples preferably within 1 hour of collection if left on the bench or within 6 hours if kept at 4°C. Blood in ACD, however, can be stored for up to 1 week but will be unsatisfactory if there is any haemolysis. Add 2 ml of blood to the tube containing 0.2 ml of the combined reagent either freshly prepared or dried. Close the tube with a stopper and gently mix the contents by inverting it 15 times. Prepare control tubes by adding 2 ml of blood to a similar tube without reagents (normal reference tube) and to a tube containing 0.1 ml of sodium nitrite-dextrose mixture without methylene blue

(“deficient” reference tube). Incubate the samples at 37°C for 90 min. If the blood has been heparinized, incubation should be continued for 3 hours. After the incubation, pipette 0.1 ml volumes from the test sample, the normal reference tube, and the deficient reference tube into 10 ml of water in separate, clear glass test tubes of identical diameter. Mix the contents gently. Compare the colours in the different tubes (Dacie and Lewis, 2018).

Interpretation

Normal blood yields a colour similar to that in the normal reference tube (i.e., a clear red). Blood from deficient subjects gives a brown colour similar to that in the deficient reference tube. Heterozygotes give intermediate reactions. Although this method takes longer than the fluorescent test, its advantages include the fact that it is extremely inexpensive and that the only equipment required is a water bath. In addition, the test can be complemented by cytochemical analysis (Dacie and Lewis, 2018).

9.5 Ascorbate Cyanide Test

This is the most sensitive screening test for detecting heterozygotes and for detecting G6PD deficiency during haemolytic attacks. The test is not specific for G6PD deficiency but also will detect other defects or deficiencies in the hexose monophosphate pathway. The test is positive in paroxysmal nocturnal haemorrhage (PNH), pyruvate kinase deficiency and in unstable Hb disorders (Glader, 2019).

Principle

The principle of the test is based on the failure of G6PD deficient cells to reduce hydrogen peroxide. Patient's blood is incubated with sodium ascorbate, sodium cyanide and glucose. Hydrogen peroxide is generated by the interaction of ascorbate with haemoglobin. Sodium cyanide inhibits the activity of normal erythrocyte catalase, an inhibitor of the formation of hydrogen peroxide. With the inhibition of catalase, hydrogen peroxide is formed. Red blood cells deficient in G6PD cannot reduce the peroxide and Hb is oxidized to methaemoglobin (Hi). Hi imparts a brown tint to the solution (Glader, 2019).

9.6 Motulsky Dye Reduction Test

In the dye reduction screening test, a haemolysate of patient's blood, G-6-P, NADP and the dye brilliant cresyl blue (BCB) are incubated together. If the haemolysate contains G-6PD, the NADP will be reduced to NADPH which in turn reduces the blue to its colourless form. The time it takes for the change to occur is inversely proportional to the amount of G6PD present (Miller, 2019).

10.0 Treatment

Patients with G-6-PD deficiency should avoid exposure to drugs and other toxic agents known to induce haemolysis (Miller, 2019, Glader, 2023). Bacterial and viral infections, diabetic ketoacidosis and hepatitis should be treated appropriately and recognized as causing haemolysis. The general principle of care including transfusion for neonatal hyperbilirubinaemia or hypoplastic crisis is similar to those for another hereditary haemolytic syndrome. Splenectomy in patients with chronic congenital haemolytic anaemia is of dubious benefit. The lack of splenic sequestration must be reconciled with the commonly held concept of splenic entrapment of Heinz body, laden, rigid erythrocytes as the primary pathogenic mechanism of haemolysis. Intravascular haemolysis and sequestration in the liver, bone marrow and other reticuloendothelial tissues occur as well. In the rare case of severe congenital haemolytic anaemia, splenectomy may be indicated. Susceptible patients from racial or ethnic groups with high gene frequency of G-6-PD deficiency should be tested prior to the administration of a potentially toxic drug (Miller, 2019). Pregnant and nursing women known to be heterozygous for the deficiency also should avoid ingestion of drugs with oxidant potential because some drugs gain access to the foetal circulation and to breast milk. If the indication for its use is sound, however, an offending drug justifiably may be given despite modest shortening of red cell survival. For example, primaquine can be safely given to individuals with the G-6-PD A- variant, provided it is started cautiously (15 mg/day or 45 mg once or twice weekly) and the blood count is monitored closely (Glader, 2019). In the term infant, phototherapy is required if the bilirubin

exceeds 170 $\mu\text{mol/l}$ during the first 2 days of life, or 240 $\mu\text{mol/l}$ on day 4 or later. Exchange transfusion is indicated if the bilirubin exceeds 250 $\mu\text{mol/l}$ during the first 2 days, or 330 $\mu\text{mol/l}$ at any time (Mehta, 2020).

Conclusion

Previous researches have indicated that G6PD deficiency is rapidly increased, which is highly attributed to poor diet in most population. There is need for the inclusion of G6PD screening as a routine test in the Laboratory and creating awareness among health professionals is very essential in this respect.

Acknowledgement: We would like to sincerely appreciate all the authors who contributed financially to the payment of publication for this manuscript. We would also especially like to thank all the authors for their constant support, direction, and encouragement.

Competing interest: All authors declare that they have no competing interests.

References

Ademowo, O.G and Falusi, A.G. (2022). Molecular Epidemiology and Activity of Erythrocyte G6PD Variants in a Homogeneous Nigerian Population. *East Africa Medical Journal*; **79**: 42-44.

Akanni, E.O., Oseni, B.S.A., Agbona, V.O., Tijani, B.A., Tosan, E., Fakunle, E.E. and Mabayoje V.O. (2020). Glucose-6-Phosphate Dehydrogenase Deficiency in Blood Donors and Jaundiced Neonates in Osogbo, *Nigeria Journal of Medical Laboratory and Diagnosis*; **1(1)**: 1-4.

Au, S.W., Naylor, C.E., Gover, L., Vandeputte-Rutten, S.D.A, Mason, P.J., Luzzatto, L., Lam, V.M.S. and Adams, M.J. (2020). Solution of the Structure of Tetrameric Human Glucose-6-Phosphate Dehydrogenase by Molecular Replacement. *Acta Crystallographica Section D. Biological Crystallography*; **55(4)**: 826-834.

Au S. W., Gover S., Lam V. M. S., Adams M. J. (2021): Human Glucose-6-Phosphate Dehydrogenase: The Crystal Structure Reveals A Structural NADP (+) Molecule And Provide Insights Into Enzyme

Deficiency. *Structure*; **8**: 293-303.

Beutke, K., Beutler, E., Brewer, G. J., Kirkman, H.N., Luzzatto, L., Motulsky, A.G., Ramot, B., Siniscalco, M. and Stanley, C.C. (2018). Standardization of Procedures for the Study of Glucose-6-Phosphate Dehydrogenase Deficiency: Report of A WHO Scientific Group. *World Health Organisation Technical Report Series*; **366**: 1-53.

Beutler, E., Dern, R.J. and Alving, A.S. (2021). The Haemolytic Effect of Primaquine, VI: an in vitro Test for Sensitivity of Erythrocytes to Primaquine. *Journal Laboratory Clinical Medicine*; **45**:40-50.

Beutler, E., Dern, R.J., Flanagan, C.L. and Alving, A.S. (2021). The Haemolytic Effect of Primaquine, VII: Biochemical Studies of Drug Sensitive Erythrocytes. *Journal Laboratory Clinical Medicine*; **45**:286-295.

Beutler, E., Mathai, C.K. and Smith, J.E. (2020). Biochemical Variants of Glucose- 6-Phosphate Dehydrogenase Giving Rise to Congenital Non spherocytic haemolytic Disease. *Blood*; **31**:131.

Beutler, E. (2020). Haemolytic Anaemia in Disorders of Red Cell Metabolism. New York. *NY: Plenum Press*; **6(4)**:8-13.

Beutler, E., Dern, R.J., Flanagan, C.L. and Alving, A.S. (2020). The red cell: a tiny dynamo *Wintrobe MM, Ed. Blood Pure and Eloquent. New York, NY: McGraw-Hill*; **2(4)**:141-68.

Beutler, E., Kuhl, W., Vives-Corrans, J.L. and Prchal, J.T. (2019). Molecular Heterogeneity of G-6-PDA-. *Blood*; **74**: 2550-2555.

Beutler, E. (2019). Glucose-6-Phosphate Dehydrogenase: New Perspective. *Blood*; **73(6)**: 1397-1401.

Beutler, E., Kuhl, W., Vives-Corrans, J.L. and Prchal, J.T. (2020). The Genetics of Glucose-6-Phosphate Dehydrogenase Deficiency. *Seminars in Haematology*; **27(2)**: 137-164.

Beutler, E. (2018). **G-6-PD Deficiency. Blood**; **84(11)**: 3613-3636.

Beutler, E. (2021). G-6-PD: Population Genetics and Clinical Manifestation. *Blood Reviews*; **10**: 45-52.

Beutler, E. (2022). Glucose-6-Phosphate Dehydrogenase Deficiency and Antimalarial Drug Development. *American Journal of*

- Tropical Medicine and Hygiene*; **77(4)**: 779-789.
- Beutler, E. (2023). Glucose-6-Phosphate Dehydrogenase Deficiency: A Historical Perspective. *Blood*; **111**: 16-24.
- Brewer, G.J., and Kellermeyer, R.W. (2017). The Haemolytic Effect of Primaquine. Shortened Erythrocyte Life Span in Primaquine Sensitive Male Negroes in the Absence of Drug Administration. *Journal Laboratory Clinical Medicine*; **58**:217-24.
- Cappellini, M.D and Fiorelli, G. (2018). Glucose-6-Phosphate Dehydrogenase Deficiency. *Lancet*; **371**: 64–74.
- Carson, P.E., Flangan, C.L., Ickes, C.E. and Alving. (2019). Enzymatic Deficiency in Primaquine Sensitive Erythrocytes. *Science Journal Laboratory Clinical Medicine*; **124**:484-5.
- Chevion, M., Navok, T., Glaser, G. and Mager, J. (2022). The Chemistry of Favism-Inducing Compounds. *European Journal of Biochemistry*; **127**: 405–409.
- Cohen, P. and Rosemeyer, M.A. (2020). Subunit Interactions of Glucose - 6- Phosphate Dehydrogenase from Human Erythrocytes. *European Journal of Biochemistry*; **8**:8-15.
- Cordes, W. (2021). Experiences with Plasmodium in MALARIA. In: anonymous. 15th Annual report. Boston, MA: United Fruit Collection; **15(7)**: 66-71.
- Dacie, C., Lewis, S. M., Bain, B. J., and Bates, I. (2006). Investigation of Hereditary Haemolytic Anaemias: Membrane and Enzyme Abnormalities: *Practical Haematology: 10th Edition Philadelphia*:217-229.
- Dern, R.J., Beutler, E. and Alving, A.S. (2015). The Hemolytic Effect of Primaquine. IV. The Relationship of Cell Age to Hemolysis. *Journal of Laboratory Clinical Medicine*. **44**:439–442.
- Dern, R.J., Beutler, E. and Alving, A.S. (2015). The Hemolytic Effect of Primaquine, V: Primaquine Sensitivity as a Manifestation of a Multiple Drug Sensitivity. *Journal of Laboratory Clinical Medicine*; **45**:30-39.
- Edward, C., Victor H., Daniel C., Edward, G. D. T., (2020). Disorders of Red Cell Metabolism *Postgraduate Haematology: 5th Edition Blackwell Publishing Ltd*: 133-150.
- Frank, E.M. (2020). Inherited Haemolytic Anaemia. *Pediatrics*; **12(5)**: 87-96.
- Gaskin, R.S., Estwick, D. and Peddi, R. (2021). G-6-PD Deficiency: it's role in the High Prevalence of Hypertension and Diabetes Mellitus. *Ethnicity and Disease*; **11(4)**: 749 – 754.
- Glader, B., John, P. G., John, F., John, N. L., George, M. R., Frixos, P., Bertil, G. (2019). **Hereditary Haemolytic Anaemias Due to Enzyme Disorders**. *Wintrobe's Clinical Haematology, 11th Edition, Lippincott Williams & Wilkins*: 908–928.
- Greene, L.S. (2021). G-6-PD Deficiency as Protection against Falciparum Malaria: An Epidemiologic Critique of Population and Experimental Studies. *American Journal of Physical Anthropology*; **36(17)**:153-178.
- Grossman, A. and Ramanathan, K. (2016). Congenital Non spherocytic haemolytic anaemia Associated with Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency in a Negro Family. *Paediatrics*; **37**:624.
- Hassan, L.G., Nasir, M. G. (2016). *An Impact Analysis of Women Economic Empowerment Policies: A Case Study of Wamakko Local Government Area Sokoto State. International Journal of Comparative Studies in International Relations and Development*; **4(1)**: 9-10.
- Hecker, P.A., Leopold, J.A., Gupte, S.A., Recchia, F.A. and Stanley, W.C. (2023). Impact of Glucose-6-Phosphate Dehydrogenase Deficiency on the Pathophysiology of Cardiovascular Disease. *American Journal of Physiological Heart Circle Physiology*; **304**: 491-500.
- Hirono, A., Kawate, K., Fujii, H. and Miwa, S. (2022). A Single Mutation 202 G>A in the Human Glucose-6-Phosphate Dehydrogenase Gene (G-6-PD) Can Cause Acute Haemolysis by Itself. *Blood*; **99**: 1498.
- Isaac, I.Z., Mainasara, A.S., Osaro, E.S.T., Omojuyigbe, M.K., Dallatu, L.S., Bilbis, T.C. A. (2013). Glucose-6-phosphate dehydrogenase deficiency among children attending the Emergency Paediatric Unit of

- Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. *International Journal of General Medicine*; **6**:557–562.
- Jidda, M. L., Ibrahim, K. K., Aiki, G., Ngaski, A. A., Blessing, J., Asiya, U. I. and Nwachukwu, C. (2017). The Prevalence of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in Students of Sultan Abdurrahman School of Health Technology Gwadabawa, Sokoto, North-Western Nigeria. *International Blood Research & Reviews*; **7**(4): 1-6.
- Julien, D., Moutawakilou, G., Joseph, A., Alphonse, N., Eurydice, M., Marcelline, S. A. and Simon, A. (2023): Prevalence of Glucose 6 Phosphate Dehydrogenase Deficiency among Infants and Children of Parakou, Benin. *Current Paediatric Resource*; **19**(12): 58-65.
- Kay, A.D. (2010). Comparison of Quantitative and Qualitative Tests for Glucose-6-Phosphate Dehydrogenase Deficiency. *America Journal of Tropical Medicine Hygiene*; **91**(4): 854-861.
- Kirkman, H.N., Wills, G.J. and Hendrickson, E.M. (2019). G6PD from Human Erythrocytes II: Sub active States of the Enzyme from Normal Persons. *Journal of Biological Chemistry*; **237**: 2371-2376.
- LaRue N., Maria K., Marjorie M., Brandon T. L., Pooja B., Sarah M., Michael K., and Gonzalo J. D. (2020): Comparison of Quantitative and Qualitative Tests for Glucose-6-Phosphate Dehydrogenase Deficiency. *America Journal of Tropical Medicine Hygiene*; **91**(4): 854-861.
- Lozano, R. (2022). Global and Regional Mortality from 235 Causes of Death for 20 Age Groups in 1990 and 2010; a Systematic Analysis for the Global Burden of Disease Study. *Lancet*; **38**(9): 2095-2128.
- Luzzatto, L.M.A. (2019). Glucose-6-Phosphate Dehydrogenase Deficiency. *The Metabolic of Inherited Disease*, New York; **6**: 2237-2265.
- Luzzatto, L. and Gordon-Smith, E.C. (2021). Inherited haemolytic anaemia. Hoffbrand, A.V., Lewis S.M., and Tuddenham In: *Postgraduate Haematology*. Arnold, London; **4**: 120-143.
- Luzzatto, L., Mehta, A. and Vulliamy, T. (2021). Glucose-6-Phosphate Dehydrogenase Deficiency in Scriver C. R., Beadet, A. L., Sly, W. S., Valle D. *The Metabolic and Molecular Bases of Inherited Disease*, 8th Edition, Columbus: McGraw-Hill, 4517-4553.
- Luzzatto, L. (2023). Glucose-6-Phosphate Dehydrogenase Deficiency: From Genotype to Phenotype. *Haematological*; **91**: 1303-1306.
- Luzatto, L. and Poggi, V. (2020): Glucose-6-Phosphate Dehydrogenase Deficiency. *Nathan and Oski's Haematology of Infancy and Childhood 7th Edition*, Philadelphia: Saunders/Elsevier: 883-909.
- Mason, P.J. (2016). New insights into G6PD deficiency. *British Journal of Haematology*; **94**: 585-91.
- Mehta, A.B. (2019). Glucose-6-Phosphate Dehydrogenase Deficiency. *Postgraduate Medical Journal*; **70**: 871–877.
- Mehta, A., Mason, P.J. and Vullaimy, T.J. (2024). Glucose-6-Phosphate Dehydrogenase Deficiency. *Bailliere's Best Practice Resource Clinical Haematology*; **13**: 21-38.
- Meletis, J., Konstantopoulos, K. and Vianny, T. (2024). Favism from the 'avoid fava beans' of Pythagoras to the present. *Haematology Journal*; **7**: 17-21.
- Meloni, L., Manca, M.R., Loddo, I. and Muntoni, S.A. (2018). Glucose-6-phosphate Dehydrogenase Deficiency Protects against Coronary Heart Disease. *Journal of Inherited Metabolic Disease*; **31**: 412-417.
- Miller, D. R., Denis R. M., Howard, A., Pearson, R. L., Baehner, C. W. (2019). Haemolytic Anaemia: Metabolic Defects. *Smith's Blood Diseases of Infancy and Childhood*. Saint Louis: 313–382.
- Monchy, D., Babin, F.X., Screy, C.T., Ing, P.N., Von Xylander, S. and Ly, V. (2024). G6PD Deficiency: Frequency in a Group of Preschool Age Children from a Central Region of Cambodia. *Medicine of Tropics*; **64**: 355-358.
- Nassef, S.R., Contini, E., and Carru, C.S. (2023). G-6-PD Deficiency in Women. *Journal of Histochemistry and Cytochemistry*; **57**(11): 1003–1011.
- Nkhoma, E. T., Poole, C., Vannappagari, V., Hall, S.A. and Beutler, E. (2021): The Global

- Prevalence of Glucose-6-Phosphate Dehydrogenase Deficiency: A Systematic Review and Meta- Analysis. *Blood Cells Molecular Disease*; **42**:267–278.
- Obasa, T.O., Mokuolu, O.A. and Ojuawo, A. (2021). Glucose-6-Phosphate Dehydrogenase Levels in Babies Delivered at the University of Teaching Hospital. *Nigeria Journal of Paediatric*. **38 (4)**: 165 - 169.
- Olatundun W., Daniel G., Grace, E., Ann, B., Tina, S., Troy, C. L. (2023). Glucose-6-Phosphate Dehydrogenase Deficiency in Nigerian Children: *Journal Pone*; **7(8)**:12-22.
- Persico, M.G., and D'Urso, M. (2016). Isolation of human glucose-6-phosphate dehydrogenase (G6PD) cDNA clones: primary structure of the protein and unusual 5' non-coding region. *Nucleic Acids Resource*; **14**: 2511-2522.
- Peters, A. L. and Van N. (2021). Glucose-6-Phosphate Dehydrogenase Deficiency and Malaria: Cytochemical Detection of Heterozygous G-6-PD Deficiency In Women. *Journal of Histochemistry and Cytochemistry*; **57(11)**: 1003–1011.
- Pinna, A., Contini, E.L. and Carru, C.S. (2023). Glucose – 6 - phosphate Dehydrogenase Deficiency and Diabetes Mellitus with Severe Retinal Complications in a Sardinian Population, Italy. *International Journal of Medical Science*; **10**:1907-1913.
- Prchal, J.T. and Xylina, T.G. (2023). Red Cell Enzymopathies. *Haematology: Churchill Livingstone*; **3**:562-576.
- Rashidi, H., Shafiei, M. and Hamidian, R. (2023). Erythrocytic glucose-6- Phosphate Dehydrogenase activity in Diabetic Patients. *Pakistan Journal of Medical Science*; **25**:665-8.
- Recols, G.J., Hatzidakis, C.J. and Schulpis, K.H. (2022). G-6-PD Deficiency in Neonatal Screening: Preliminary Evidence that High Percentage of Partially Deficient Female Neonate are Missed During Routine Screening. *Journal of Medical Screen*; **7**:46-51.
- Scopes, D., Bautista, J.M., Naylor, C.E., Adams, M.J. and Mason, P.J. (2018). Amino Acid Substitutions at the Dimer Interface of Human Glucose-6-Phosphate Dehydrogenase that Increase Thermostability and Reduce the Stabilising Effect of NADP. *European Journal of Biochemistry*; **251**: 382-388.
- Segel G.B. (2022): Enzymatic defects. *Nelson Textbook of paediatrics*, Philadelphia; **16**:1488-1491.
- Shirish, M. and Kawthalkar, S. (2018). Disorders of Red Cell Enzymes. *Essentials of Haematology. Jaypee Brothers. Medical Publishers (P) Limited, New Delhi*: 183–190.
- Singh Siddhartha, AshaAnand and Pramod K. Srivastava (2023). Regulation and Properties of Glucose-6-Phosphate Dehydrogenase; A Review. *International Journal of Plant Physiology and Biochemistry*; **4(1)**: 1-19.
- Simoons, F.J. (2018). Plants of Life, Plants of Death. *The University of Wisconsin Press*, 216–249.
- Sterling, K., Gray, S.J. and Pramod, K.S. (2017). Determination of the circulating red cell volume in man by radioactive chromium. *Journal of Clinical Investigations*; **29**:4-9.
- WHO Working Group (2020). Glucose-6-Phosphate Dehydrogenase Deficiency. *Bulletin World Health Organisation*; **67**: 601–611.
- Wrigley, N.G., Heather, J.V., Bonsignore, A. and De Flora, A. (2017). Human Erythrocyte Glucose 6-Phosphate Dehydrogenase: Electron Microscope Studies on Structure and Interconversion of Tetramers, Dimers and Monomers. *Journal of Molecular Biology*; **68**: 483-499.
- Vives-Corrons, J.L., Kuhl, W., Pujades, M.A., and Beutler, E. (2017). Molecular Genetics of the Glucose-6-Phosphate Dehydrogenase (G-6-PD) Mediterranean Variant and Description of a New G-6-PD Mutant, G-6-PD Andalus. *American Journal of Human Genetic*; **45**: 575-579.
- Vulliamy, T. J., Urso, M.D., Battistuzzi, G., Estrada, M., Foulkes, N. S., Martini, G., Calabro, V., Poggi, V., Giordano, R., Town, M., Luzzatto, L. and Presico, M. G. (2019). Diverse Point Mutations in the Human Glucose-6-Phosphate Dehydrogenase Gene cause Enzyme Deficiency and Mild to Severe Haemolytic Anaemia. *Proceedings of the*

National Academy of Science USA; **85(14)**: 5171-5175.

Xavier, M.B. (2022). G6PD deficiency: the general practitioner's role in the screening and prevention of crises [Doctoral Thesis in Medicine]. France: Faculty of Medicine of Marseille. *Journal for Clinical*

Haematology; **150(9)**: 44-65.

Zaffanello, M., Rugolotto, S., Zamboni, G., Gaudino, R., and Tato, L. (2024). Neonatal Screening for Glucose-6-Phosphate Dehydrogenase Deficiency Fails to Detect Heterozygous Females. *European Journal of Epidemiology*. **19(3)**: 255-257.

Citation: Ibrahim Kalle Kwaifa, Osaro Erhabor, Abdulrahman Yakubu, Aliyu Ibrahim Bagudo, Buhari Hauwa Ali, Onuigwe Festus Uchechukwu, Nura Muhammad Bunza, Mustapha Umar Kalgo, Abdullahi Isiyaku, Mukhtar Yeldu, Dahiru Isah, Moses Akila, Solomon Moses, Zainab Abubakar Ibrahim, Ukashat Almustapha, Muhammad Lubabatu Abbas, Addra Sumaiya Ibrahim, Stephen Cecilia, Ismail Usman Adenkunle, Nasiru Sadiq, and Muhammad Buhari. Pathophysiology and Current Laboratory Investigations of Glucose-6-Phosphate Dehydrogenase Deficiency. *Sokoto Journal of Medical Laboratory Science*; **10(1)**: 358–379. <https://dx.doi.org/10.4314/sokjmls.v10i1.35>.

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.