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Glucose-6-phosphate dehydrogenase : distribution of the African form in the Arab and Mediterranean countries

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List of Abbreviations

3-DPG:	2,3-diphosphoglycerate
6-PG:	6- Phosphogluconate
PGD:	Phosphogluconic dehydrogenase
6-PGD:	6-Phospho gluconic dehydrogenase
AHA:	Acute hemolytic anemia
CDH1:	Cadherin-1
CNSH:	Chronic non spherocytic hemolytic
CoA:	Coenzyme A
COVID-19:	Corona virus diseases 2019
cRNA:	Complementary RNA
CVD:	Cardio vascular diseases
EMT:	Epithelial-Mesenchymal Transition
FAD:	Flavine adenine dinucleotide
G-3-PD:	Glyceraldehyde phosphate dehydrogenase
G6P:	Glucose-6-phosphate
G6PD:	Glucose-6-Phosphate dehydrogenase
GPX:	Glutathione peroxidase
MERS-COV:	Middle East respiratory syndrome corona virus
NADH:	Nicotinamide adenine dinucleotide
NADHP:	Nicotinamide adenine dinucleotide Phosphate
PAGE:	Polyacrylamide Gel Electrophoresis
PPP:	Pentose Phosphate Pathway
RFLP:	Restriction Fragment Length Polymorphism
ROS:	Reactive Oxygen Species
SARS-COV:	Severe acute respiratory syndrome coronavirus
UDPG-T:	Uridine diphosphate glucuronosyl transferase

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect being present in more than 400 million people worldwide (Cappellini and Fiorelli, 2008), it's common in populations living in malaria-endemic areas (Carter et al., 2011). It was discovered in the 1950s as a result of investigations into self-limited hemolysis that occurred after administration of the antimalarial drug primaquine, most commonly in individuals of African or Mediterranean ethnic origin (Gregg and Prchal, 2018).

G6PD is the rate-limiting enzyme in the pentose phosphate pathway. G6PD converts glucose-6-phosphate (G6P) into 6-phosphogluconolactone with the concomitant production of nicotinamide adenine dinucleotide phosphate (NADPH), a reducing equivalent necessary for reductive biosynthesis and redox homeostasis (Salati and Amir-Ahmady, 2001).

The clinical manifestations of G6PD deficiency include jaundice, favism, and drug or infection-induced hemolytic anemia (Noori and Daneshpajooh, 2008 ; Mason, 2015 ; Muzaffar *et al*., 2017).

G6PD deficiency represents one of the most common inherited and sex-linked enzymopathies. The G6PD gene maps to the X-chromosome; thus, the phenotype is manifest fully in males whereas female heterozygotes display varying degrees of G6PD deficiency, due to alternate X-chromosome inactivation (Cappellini and Fiorelli, 2008 ; Belfield and Tichy, 2018).

G6PD deficiency is also associated with other chronic diseases such as cardiovascular disease, malaria, and the COVID-19 pandemic (Beutler, 1994 ; Mason, 2007 ; Mbanefo et al., 2017 ; Aydemir *et al.*, 2020).

The main objective of this report was to determine the local prevalence of the African form of G6PD deficiency in some Arabic and Mediterranean countries.

Studies determined that G6PD deficiency is X-linked and subsequent studies in carrier females led to the discovery of X-inactivation, a phenomenon that has been exploited to study the hierarchy of hematopoiesis and the clonality of malignant neoplasms (Gregg and Prchal, 2018).

G6PD deficiency increases the vulnerability of erythrocytes to oxidative stress. Clinical presentations include acute hemolytic anemia, chronic hemolytic anemia, neonatal hyperbilirubinemia, and an absence of clinical symptoms. The disease is rarely fatal (Frank, 2005).

I.1. Historic

It was not until the middle of the 19th century that a Portuguese medical journal published the observation of a patient with jaundice flares every time he ate beans (Pereira de Mira, 1843).

In the middle of the 1900s, drug companies noticed that some people given primaquine, a drug used to treat malaria, became very anemic (Baker, 2013).

In the year 1926, Cordes reported the occurrence of acute hemolysis in individuals treated for malaria with primaquine drugs, however, the mechanism of hemolysis could not be understood for the next three decades (Cordes, 1926).

Three decades passed before the mechanism of hemolysis could be understood. The discoveries that led to the recognition of G6PD deficiency were the result of several convergent events (Beutler, 1980).

Primaquine used as a prophylactic treatment of malaria, during operations in Southeast Asia by World War II by the United States military, was implicated in the occurrence of acute anemia among black soldiers by Hockwald in 1952 (Baker, 2013).

In 1956, Dr.Carson’s team published the results of their research which identified that the enzyme G6PD deficiency is the cause (Baker, 2013).

In 1958, Childs detected the genetic anomaly responsible on the X chromosome, explaining why the transmission of this deficit mainly affects men. Since then, research has

led to a better understanding of the genetic, molecular, and pathophysiological aspects involved (Beutler, 1959).

In 1959, Beutler described the biochemical mechanism of hemolytic anemia after taking oxidative drugs (Beutler, 1959).

In 1962, the recognition of the strict biochemical analogy of the erythrocytes in favism and primaquine sensitivity (Sartori, 1971).

In 1966, the world health organization (WHO) assembled a working group to study this disease and its different variants. Over the next 20 years, it is believed to discover around 400 biochemical variants (Cappellini and Fiorelli, 2008).

In 1967, the first lists of harmful drugs, including sulfonamides, were published (Cappellini and Fiorelli, 2008).

In 1986, Persico et al. and Tettakizawan et al. have cloned G6PD and have sequenced the G6PD gene (Cappellini and Fiorelli, 2008).

In 1989, WHO published in its bulletin the first synthetic study of this deficit, carried out with the assistance of biochemists, hematologists, and pediatricians. This study describes the aspects, the global geographic distribution, the polymorphism, the mechanisms, and the prevention of the disease (WHO working group, 1989).

In 1996, the development of the three-dimensional human G6PD model (Naylor *et al.*, 1996).

I.2. Epidemiology

G6PD deficiency occurs with increased frequency throughout Africa, Asia, the Mediterranean, and the Middle East. In the United States, black males are most commonly affected, with a prevalence of approximately 10 percent. The prevalence of the deficiency is correlated with the geographic distribution of malaria, which has led to the theory that carriers

of G6PD deficiency may incur partial protection against malarial infection. Cases of sporadic gene mutations occur in all populations (Frank, 2005).

Also is one of the most prevalent genetic diseases in Arab countries; it is reported to have a high prevalence in Saudi Arabia (39.8%), Syria (30%), and Oman (29%) compared to other Arab countries. More than 300 different mutations have been reported in the G6PD gene. The Mediterranean mutation is the most prevalent among Arabs, with 90% frequency in Bahraini patients, 87.8% in Northern Iraqi males, 74.2% in Kuwait, and 53.6% in Jordan (Doss *et al.,* 2016).

I.3. Clinical manifestation

I.3.1. Acute hemolytic anemia

The acute hemolytic anemia (AHA) is the most common manifestation of the deficiency, which is originated when the red blood cells (RBC) are under oxidative stress and may be triggered by a range of exogenous agents as foods (e.g fava beans), drugs, or infections (Gómez-Manzo *et al.,* 2016). Malaise, abdominal, low back pain, and hemoglobinuria, the passing of red or dark urine, are usually the accompanying symptoms (Frank, 2005).

I.3.1.1. Drugs-induced hemolysis

G6PD deficiency was first discovered by investigating hemolysis that had developed in patients who had received primaquine. Successively, several drugs were associated with acute hemolysis in G6PD-deficient patients. To decide if a specific drug can directly cause a hemolytic crisis in G6PD-deficient patients is often challenging to establish (Muzaffar *et al.,* 2018).

I.3.1.2. Infection-induced hemolysis

Infection is perhaps the most usual cause of hemolysis in children with G6PD deficiency. The degree of hemolysis can be influenced by many factors, counting concomitant drug administration, age, and liver function (Muzaffar *et al.,* 2018).

I.3.2. Favism

Favism is the name given to acute hemolytic anemia brought on by eating fava beans (Mason *et al.,* 2007). Fava beans are a staple food in many parts of the world where G6PD

deficiency is found at a high gene frequency. The hemolysis precipitated by fava bean ingestion, favism, occurs only in people who are G6PD deficient. It is most frequently associated with the more severe G6PD Mediterranean and G6PD Cairo variants but rarely has been seen with G6PD A-Not all individuals with G6PD Mediterranean are susceptible to favism and a tendency toward familial occurrence suggests that additional genetic factors may be important. Favism is more common in children than in adults. Hemolysis usually occurs one to several days after fava bean consumption, but onset within the first hours after exposure has been reported (Gregg and Prchal, 2017). Favism can even occur in the breast-fed infant whose mother had ingested fava beans (Noori-Daloii, 2009).

I.3.3. Neonatal jaundice

The principal cause of neonatal icterus in G6PD-deficient infants is the inability of the liver to adequately conjugated bilirubin. This problem is compounded when the infant also inherits the glucuronosyltransferase promoter polymorphism (UDP) that is associated with Gilbert disease (Kaplan *et al.,* 1997). In the neonatal period, the major manifestation of G6PD deficiency is hyperbilirubinemia. Jaundice is not typically present at birth, with clinical onset between day 2 and day 3 (Kaplan *et al.,* 2016). Most infants with hyperbilirubinemia caused by G6PD deficiency are of Mediterranean, Middle Eastern, or Asian descent (Christensen, 2018). The mechanism by which G6PD deficiency causes neonatal hyperbilirubinemia is not fully understood. Although hemolysis may be observed in neonates who have G6PD deficiency and are jaundiced (Nouri and Danesh, 2008).

I.3.4. Chronic hemolysis

A small number of individuals have a very severe deficiency of G6PD with ongoing hemolysis in the absence of triggering factors. They have mild-to-moderate anemia (Hb 8–10 g/dl) and reticulocytosis of 10–15% (Mckew *et al.,* 2013). Another manifestation of G6PD deficiency was found to be hereditary nonspherocytic hemolytic anemia. This syndrome, first delineated by William Crosby in 1950 (Beutler, 2008). In some patients, variants of G6PD deficiency cause chronic hemolysis, leading to so-called congenital non-spherocytic hemolytic anemia. These variants have been grouped as class 1 in the proposed WHO classification. The G6PD variants causing congenital non-spherocytic hemolytic anemia are all sporadic, and almost all arise from independent mutations (Cappellini and Fiorelli, 2008).

I.4. Triggers

Antibiotics: Sulphonamides (such as sulfanilamide, sulfamethoxazole, and mafenide), Cotrimoxazole (Bactrim, Septrin). Dapsone, Chloramphenicol, Nitrofurantoin, Nalidixic acid, Sulfisoxazole, Sulfamethoxazole/trimethoprim, Isoniazid (Devi *et al.*, 2016).

Antimalarials: Chloroquine, Hydroxychloroquine, Primaquine, Pamaquine, Quinine, Mepacrine (Devi *et al*., 2016).

Chemicals: Moth Balls (naphthalene), Methylene blue (Devi *et al.*, 2016).

Analgesics: Aspirin , Acetaminophen , Phenazopyridine (Devi *et al.*, 2016).

Other drugs: Sulphasalazine, Methyldopa, Large doses of vitamin C, Hydralazine, Thiazolesulfone, Procainamide, Quinidine, Dimercaprol, Glyburide, Rasburicase, Prilocaine, Vitamin K (water-soluble derivatives), Some anti-cancer drugs, Henna has been known to cause a hemolytic crisis in G6PD-deficient infants, Herbs like Coptis Chinensis and Calculus Bovis (Devi *et al*., 2016).

Infections: Infection is a cause of hemolysis in the G6PD deficient subject : *Salmonella, Escherichia-coli* infections, *β-hemolytic streptococcus, Rickettsioses,* viral hepatitis. Hepatitis A, Malaria, *Pneumococcal pneumonia* (Sharma *et al.,* 2018 ; Aubry and Gaüzère, 2020).

Food: ingestion of fava beans (Aubry and Gaüzère, 2020).

I.5. Association with other diseases

I.5.1. Malaria

The malaria hypothesis that the high incidence of G6PD deficiency has arisen because G6PD-deficient alleles confer some resistance against severe malaria caused by infection with *P. Falciparum* (Beutler, 1994) *Plasmodium falciparum* is responsible for 67% of malaria cases and *Plasmodium vivax* for 30%; there is a small percentage of cases due to mixed infections (Tseghereda *et al.,* 2018), the female heterozygous genotype showed a highly significant association with protection from malaria. Unlike in homo/hemizygous, also the hemizygous males are not only unprotected from malaria but maybe at high risk of severing malaria. This is because G6PD-deficient red blood cells are prone to early destruction by oxygen free radicals (Mbanefo *et al.*, 2018).

I.5.2. Cardiovascular disease

Although human cardiovascular disease (CVD) is due to multifactorial genetic and environmental conditions, a recent report has suggested that G6PD deficiency in mice may provide some protection against pressor and hypertrophic vascular response by decreasing the contribution of NADPH oxidase (Mason *et al*., 2007).

A recent meta-analysis and epidemiological studies have shown that affected individuals can have an increased risk of developing diabetes and CVD. Various G6PD deficiency models have shown the principal role of G6PD deficiency in altering redox homeostasis and the progression of CVD, which predisposes the system to defective vasodilation (Parsanathan and Jain, 2020).

I.5.3. COVID-19

The coronavirus (COVID-19) pandemic has become today's most important world public health problem. COVID-19 is an enveloped and single-stranded RNA beta-coronavirus which is very similar to severe acute respiratory syndrome coronavirus (SARS-CoV)/SARS and Middle East respiratory syndrome coronavirus (MERS-CoV)/MERS. Those at particular risk have been the elderly and those with chronic diseases including diabetes, cardiovascular diseases, cancer, and chronic respiratory diseases. These people appear to be at a greater risk of dying from COVID-19 (Aydemir and Ulusu, 2020).

Previous studies have confirmed that virus infection induces production of both reactive oxygen species (ROS) and reactive nitrogen species (RNS), both can damage proteins, DNA, and cellular components of cells when antioxidant enzyme metabolism is impaired. Since G6PD deficiency results in the redox imbalance in the erythrocytes leading to hemolysis and tissue damage as a result of insufficient oxygen transportation, COVID-19 might increase the mortality risk of patients with G6PD deficiency, the relationship between G6PD deficiency and COVID-19 infection is unknown. Although mortality risk is higher for older adults and individuals with chronic health conditions, G6PD enzyme deficiency should be taking into consideration in the risk assessment for the COVID-19 pandemic (Aydemir and Ulusu, 2020).

The association between G6PD deficiency and novel coronavirus disease is absent in COVID-19 reports. This absence could be because G6PD deficiency was overlooked during the current COVID-19 pandemic. One may argue that using hydroxychloroquine to treat

COVID-19 was less likely, as hydroxychloroquine needs to be used cautiously in G6PD deficiency. Another reason for this absence could be that G6PD deficiency in the countries most affected by COVID-19 is rare and/or of the mild type (Al-Abdi and Al-Aamri, 2020). Hydroxychloroquine has been proposed as a treatment for COVID-19 and clinical trials have been started evaluating this proposal. Hydroxychloroquine has oxidative properties that could decrease glutathione levels and may cause severe hemolysis in G6PD-deficient patients. If hydroxychloroquine is found to be the silver bullet for COVID-19, then this may be a big challenge in treating COVID-19 in G6PD-deficient patients (Al-Abdi and Al-Aamri, 2020).

II.1. Physiopathology and mechanism of G6PD deficiency

II.1.1. G6PD Enzyme structure

The G6PD monomer consists of 515 amino acid subunits with a molecular weight of 59,256 daltons. The active enzyme exists as a dimer and contains tightly bound NADP. Aggregation of the inactive monomers into catalytically active dimers and higher forms requires the presence of NADP. Thus, NADP appears to be bound to the enzyme both as a structural component and as one of the substrates of the reaction (Beutler, 1994).

The enzyme is active as a tetramer or dimer, in a pH-dependent equilibrium. Every monomer consists of two domains: the N-terminal domain (amino acids 27–200), with a β–α–β dinucleotide binding site (amino acids 38–44); and a second, larger, β+α domain, consisting of an antiparallel nine-stranded sheet. The dimer interface lies in a barrel arrangement, in this second part of the G6PD molecule. The two domains are linked by an α helix, containing the totally conserved eight-residue peptide that acts as the substrate-binding site (amino acids 198–206). Viewing the structure, at 3 Å (0,3 nm) resolution, reveals an NADP+ (a coenzyme) molecule in every subunit of the tetramer, distant from the active site but close to the dimer interface (Fig. 1). Stability of the active quaternary structures is crucial for normal G6PD activity (Fiorelli *et al.,* 2000 ; Cappellini *et al.,* 2008).

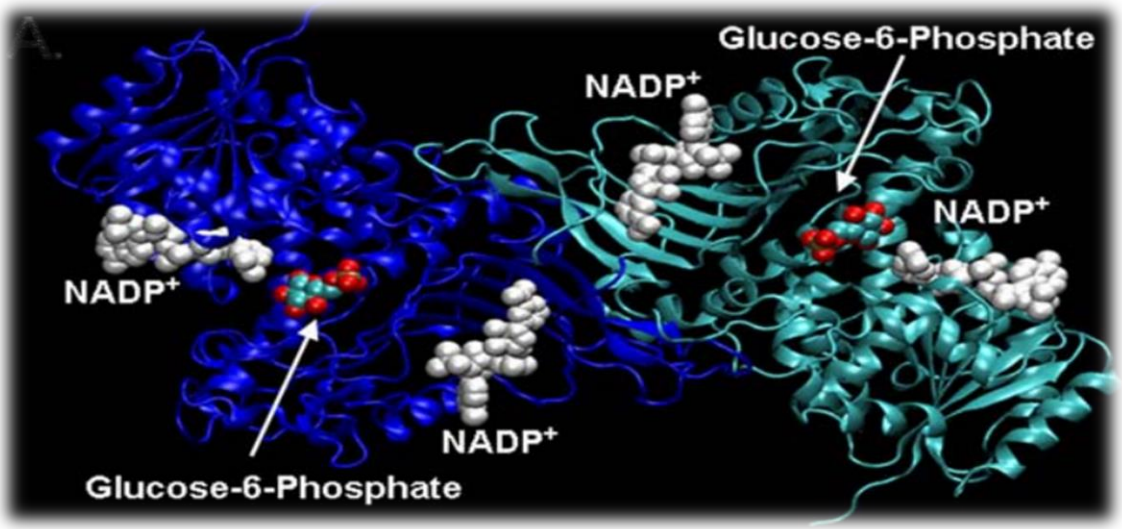

Fig. 1. Wild-type structure of the human G6PD, with NADP+ entries from 2BH9 and G6P entries from 2BHL (shown as van der Waals representation). The two chains are shown in green and blue (Kiani *et al*., 2007).

II.1.2. Role of G6PD enzyme

ROS cause oxidative stress and damage the cell's membranes. Increased production of ROS or decreased antioxidant defense enzymes play a major role in oxidative injuries in different organs, tissues, and cells including the brain, heart, vascular cells and causes brain diseases like Alzheimer's and Parkinson's diseases and also considered to contribute to the aging process and because G6PD is a housekeeping enzyme, expressed in all cells of the body (Luzzatto *et al*., 2016). It has an important role in all cells as an antioxidant defense enzyme especially in red blood cells (Farhud and Yazdanpanah, 2008).

Studies in knockout mice reveal that G6PD plays a fundamental role in embryonic development. However, the detailed mechanism regarding why its knockout is embryonically lethal has not been clearly elucidated. The evidence obtained in the current study indicates that G6PD plays an important role in embryonic development, partly by affecting the expression of adhesion molecules. G6PD knockdown in zebrafish can cause epithelial-mesenchymal transition (EMT) or adhesion defects during embryonic development, whereas Cadherin-1 complementary RNA (CDH1 cRNA) coinjection can rescue such phenotype (Wu *et al*., 2018).

G6PD is a common feature for regulating cell-cell interactions by modulating the expression of adhesion molecules. The current study also indicates that G6PD plays a cytoregulatory role in redox signaling by influencing the NOX pathway, which is critical to many cellular functions (Fig. 2) (Wu *et al*., 2018).

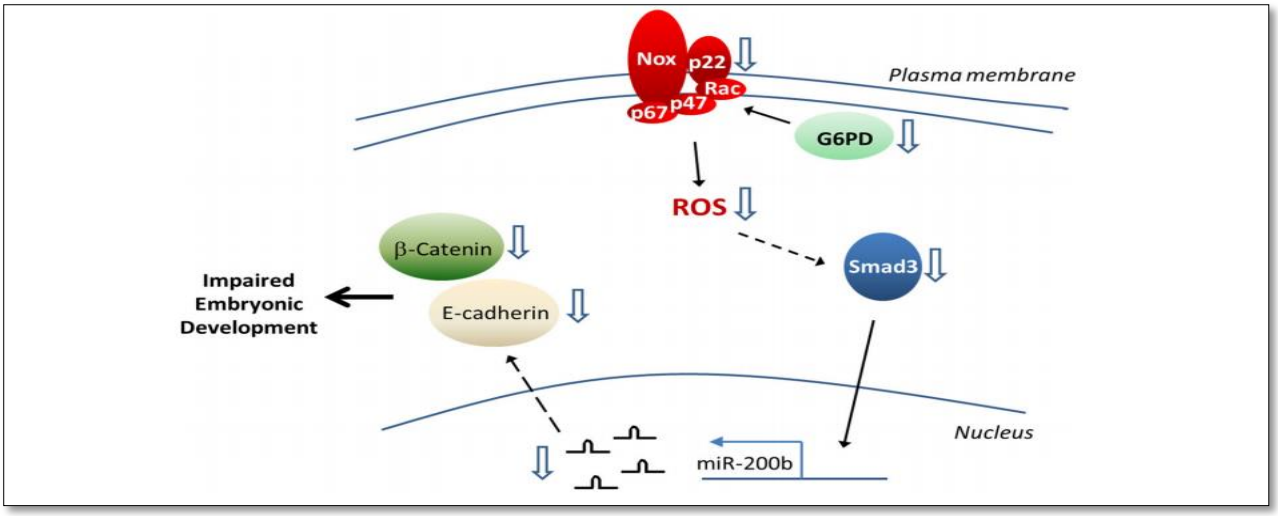

Fig. 2. Schematic depicting how G6PD status modulates the expressions of adhesion molecules, E-cadherin, and β-catenin, which play essential roles in regulating embryonic development (Wu *et al.*, 2018).

II.1.3. Function of G6PD enzyme in the pentose phosphate pathway from red blood cells

In the RBC, G6PD action is the first and rate-limiting step in the pentose phosphate pathway (PPP) that converts NADP into reduced NADPH (Aboud, 2008). PPP, also called the hexose monophosphate shunt (Howes *et al.*, 2013). Unlike other cell types, RBCs do not contain mitochondria and therefore the PPP pathway is the only source of NADPH which plays a key role in protecting cells against oxidative damage due to reactive oxygen species (ROS) (Gómez-Manzo *et al.*, 2016).

As a first step G6PD acts on glucose-6-phosphate (G6P) to produce 6-phosphoglucono-d-lactone, which in turn produces ribose-5-phosphate (R5P) via 6-phosphogluconate and ribulose-5-phosphate. These reactions can be summarised as follows :

$$G6P + 2\ NADP^{+} + H_2O \longrightarrow R5P + 2\ NADPH + 2\ H^{+} + CO_2$$

Ribose sugars are required as precursors in the biosynthesis of some important molecules, such as ATP, CoA, NAD, FAD, RNA, and DNA. Besides, the ribose-5-phosphate formed by the PPP can be completely converted back into glycolytic intermediates (Mehta *et al.*, 2000). Because the important function of NADPH is scavenging cellular ROS, NADPH is

involved in at least three antioxidant pathways: the glutathione, thioredoxin, and glutaredoxin cycles (Fig. 3). In the first pathway, the electron of NADPH passes to glutathione dimers (GSSG) during the reaction catalyzed by glutathione reductase enzyme that produces two reduced glutathione monomers (GSH) providing the first line of defense against ROS. Moreover, glutathione peroxidase (GPX) removes peroxide from RBCs using GSH as a substrate, while the NADPH is required to reduce GSSG oxidized and the sulfhydryl groups of some necessary proteins for the protection against oxidative stress. The RBCs that cannot eliminate this stress suffer of hemolysis (Gómez-Manzo *et al.*, 2016).

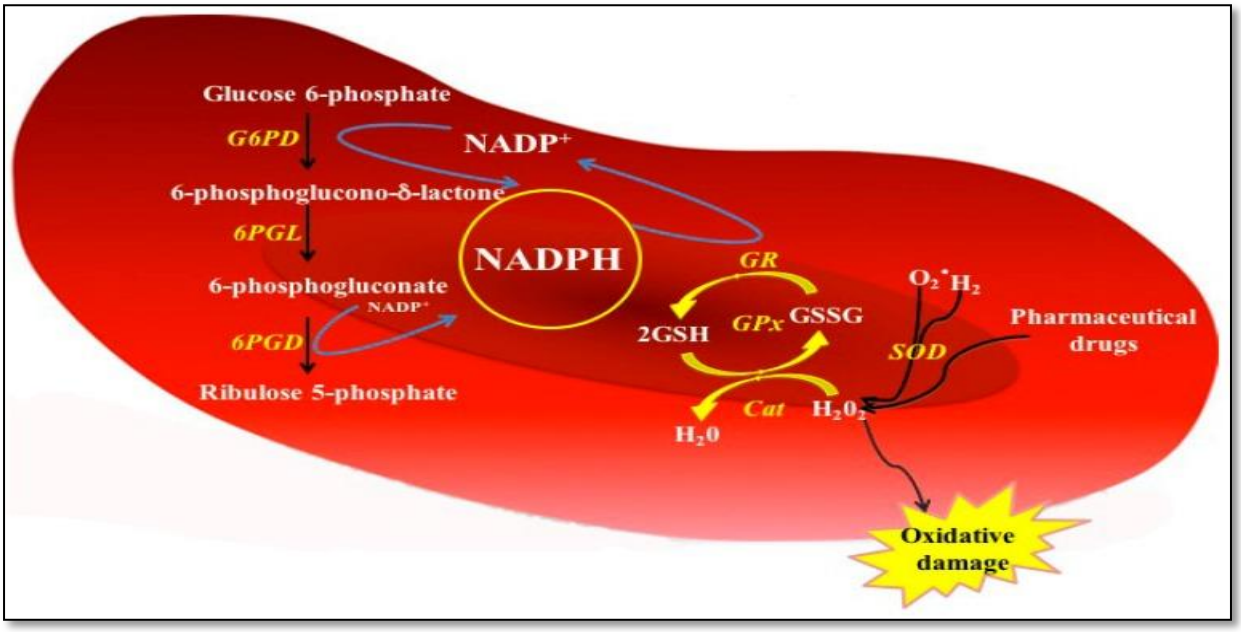

Fig. 3. Function of G6PD enzyme in the PPP from red blood cells (Gómez-Manzo et al., 2016).

II.1.4. Mechanism of hemolysis

However, in cells that have a mutant and defective G6PD gene, the PPP may, depending upon the extent of the enzyme activity defect, function at a near-maximum rate even at steady-state redox equilibrium. When an oxidative challenge occurs and the equilibrium of NADP to NADPH shifts to the oxidized direction, the PPP is intrinsically unable to accelerate rapidly enough to force the equilibrium in favor of NADPH. This effectively stymies the flow of electrons to GSH and that equilibrium shifts in favor of GSSG. The oxidants consuming these reducing equivalents, in turn, overwhelm the ability of the cell

to provide them, and damage may then occur. Visible evidence of such occurs in the form of Heinz bodies (Fig. 8) in the RBC membrane that attends acute primaquine-induced hemolytic anemia. Heinz bodies cause the membrane to become rigid, and thus decrease the cells' lifespans (Howes *et al*., 2013).

The exact mechanism where by increased sensitivity to oxidative damage leads to hemolysis remains to be established (Fang *et al*., 2016). Most is known about favism, in which the compounds divicine and isouramil, found in fava beans, have a causal role in the irreversible oxidation of GSH and other protein-bound SH groups. Effects resulting from this include electrolyte imbalance, membrane cross- bonding, and erythrocyte phagocytosis. A striking observation in favism is the increase in red cell calcium levels, and in some cases the degradation of erythrocyte calcium ATPase. A combination of both increased passive permeability and a decreased efficiency of the calcium pump may explain the disruption of erythrocyte calcium homeostasis. G6PD-deficient red cells are also more susceptible to calcium-induced vesiculation than normal cells, and this is correlated with the extent of complement-mediated hemolysis (Mehta *et al*., 2000).

II.2.Genetic of G6PD deficiency

II.2.1. G6PD gene structure

The G6PD gene is positioned at the telomeric region of the long arm of the X chromosome (Xq28), near the genes for hemophilia A, color blindness, and congenital dyskeratosis (Muzaffar *et al*., 2017)**.** The gene contains 13 exons and is over 20 Kb in length. The first exon contains no coding sequence and the intron between exons 2 and 3 is extraordinarily long, extending for 9,857 bp (Fig. 4) (Beutler, 1994). Males are more likely than females to suffer all X-linked genetic conditions (Farhud and Yazdanpanah, 2008).

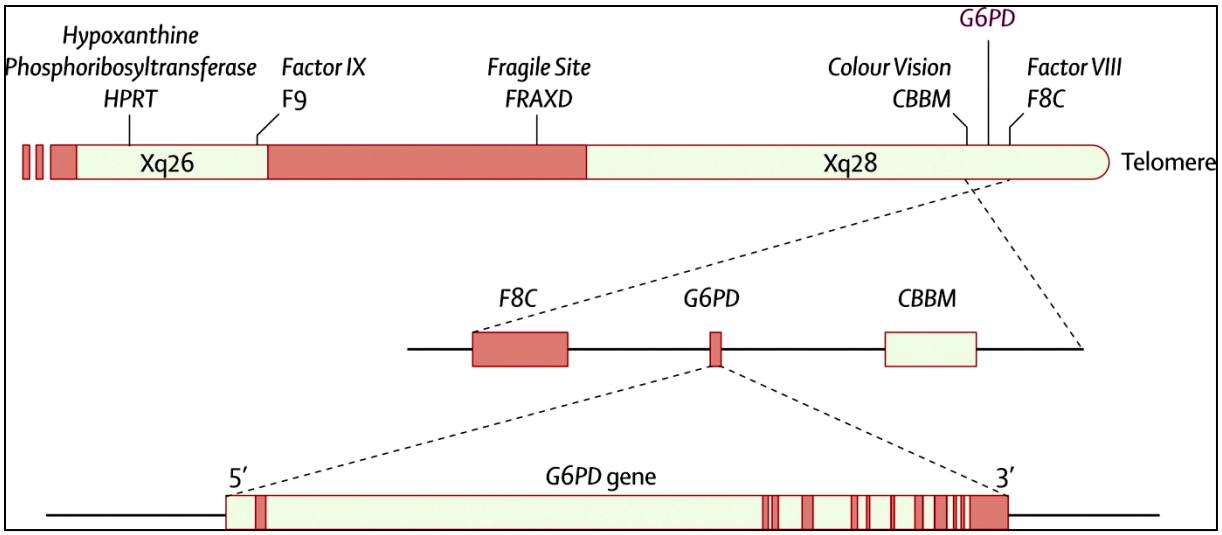

Fig. 4. Location of the G6PD gene on X chromosome (Cappellini and Fiorelli, 2008).

II.2.2. G6PD genotypes

Studies of the genetics of human G6PD variants have contributed to the understanding of G6PD deficiency and more general aspects of human genetics. G6PD deficiency is inherited as an X-linked trait, as are hemophilia and color blindness. If the X-chromosome carrying an abnormal G6PD allele is designated X*, then the three possible genotypes containing X are (Bhagavan and Chung-Eun, 2011) :

1. X*Y-hemizygous male, with the full phenotypic expression of the abnormal allele.

2. XX*-heterozygous female, with a clinically normal phenotype despite the abnormal allele expressed in about half her cells.

3. X*X*-homozygous female, with the full phenotypic expression of the abnormal allele. Sons of affected males are usually normal (because they receive their X-chromosome from their mothers), and daughters of affected males are usually heterozygotes (because they receive one X-chromosome from their father). The rarest genotype is that of the homozygous female since it requires that both parents have at least one abnormal X-chromosome (Bhagavan and Chung-Eun, 2011).

Females heterozygous for a G6PD variant are phenotypic mosaics. They have two erythrocyte populations, one containing normal G6PD, the other the variant. In fact, in

heterozygotes, every tissue has some cells expressing the normal, and some the abnormal, G6PD gene. Random X-chromosome inactivation early in embryonic development causes only one of the two X-chromosomes to be active (Bhagavan and Chung-Eun, 2011).

II.2.3. Inheritance of G6PD deficiency

Transmission occurs from mother to son but not from father to son. Male hemizygotes (XY) and female homozygotes (XX) are invariably more severely affected than female heterozygotes (XX). Heterozygous females usually have levels of G6PD intermediate between those of fully affected males and normal subjects. Some heterozygotes have normal red cell G6PD activity: others have a quantitative deficiency as severe as hemizygotes (Fig. 5). The behavior of the X-linked gene in G6PD deficiency bears out the hypothesis of X inactivation. The variable amount of total G6PD activity and the double population of cells or mosaicism in heterozygous females are determined by inactivation of the X chromosome bearing either the normal or the deficient gene for G6PD (Aboud, 2012).

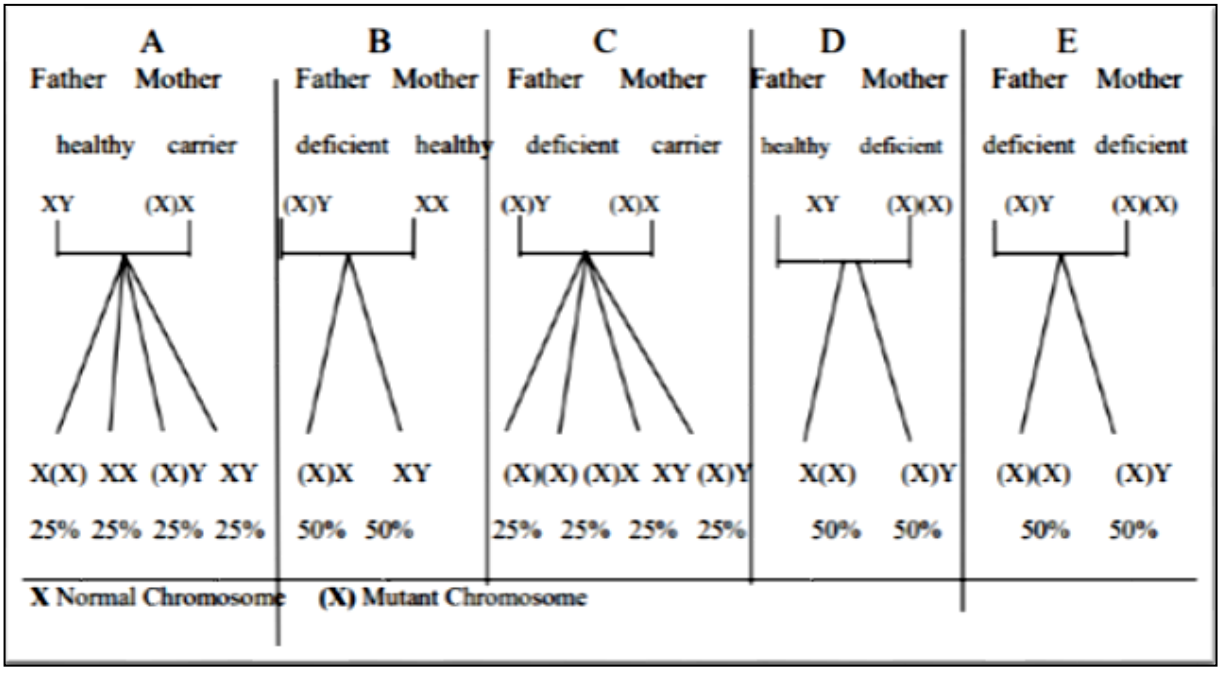

Fig. 5. Inheritance of G6PD Deficiency (Aboud, 2012).

II.2.4. G6PD variants

Variants of G6PD deficiency were grouped into five classes based on enzyme activity and clinical manifestations. Variants can also be classified as sporadic or polymorphic. The G6PD enzyme deficit can be caused by a reduction in the number of enzyme molecules, a

structural difference in the enzyme causing a qualitative change, or both. Examination of G6PD variants shows that, in most cases, G6PD deficiency is due to enzyme instability, implying that amino acid substitutions in different locations can destabilize the enzyme molecule (Howes *et al.*, 2013).

To date, more than 400 G6PD variants have been identified, of which 186 variants are associated with G6PD deficiency by decreasing the activity or stability of G6PD (Ghebremedhin *et al*., 2018).

II.2.4.1. Biochemical variants

G6PD variants are divided into five classes by the WHO scientific group, by their activity in red cells and their associated clinical manifestations on the standardization of procedures for the study of glucose-6-phosphate dehydrogenase in 1967 (Yoshida *et al.*, 1971) :

Class I: Severe enzyme deficiency with chronic non-spherocytic hemolytic anemia.

Class II: Severe enzyme deficiency (<10% of normal).

Class III: Moderate to a mild enzyme deficiency (10-60% of normal).

Class IV: Very mild or no enzyme deficiency (60-100,' of normal).

Class V: Increased enzyme activity (more than twice normal).

II.2.4.2. Polymorphic variants

Polymorphic G6PD variants, also known as WHO class II and III, have gene frequencies 1–70% in particular populations. Different geographical areas have different sets of polymorphic variants (Noori-Daloii *et al*., 2009). Those variants have achieved a high frequency in some populations. However, in sub-Saharan Africa, three variants occur with polymorphic frequencies (> 0.1%) (Ghebremedhin *et al.*, 2018) :

- G6PD*B is the wild type and the most common variant in Africa and worldwide.
- G6PD*A has a single A→G substitution at nucleotide number 376. It is a normal variant with about 90% of the G6PD*B enzyme activity.
- G6PD*A- is a deficient variant with about 8-20% of the wild type enzyme activity. G6PD A- variant is a common G6PD variant among Africans.

This class-3 phenotype can be caused by a combination of the common 376A>G (Asn126Asp) mutation and either of 3 additional mutations: 202G>A (Val68Met), 680G>T (Arg227Leu), or 968T>C (Leu323Pro). The missense mutation 376A>G by itself causes an asymptomatic class-4 variant G6PD A with normal enzyme activity, whereas the other mutation 202G>A has never been found in humans by itself. Some investigators insisted that both mutations in G6PD A- are necessary to produce the G6PD-deficient phenotype (Hirono *et al.*, 2002).

G6PD Mediterranean variant is caused by the C563T mutation with less than 10% enzyme activity and found in Italy, Cyprus, and the Middle East **(**Ghebremedhin *et al.*, 2018).

II.2.4.3. Sporadic variants

Sporadic variants causing Chronic None Spherocytic Hemolytic Anemia (CNSHA), also known as WHO class I, occur at a very low frequency in any part of the world (Fiorelli, 2000). The majority of mutations are single amino acid changes but many in-frame deletions have been found. The absence of frameshift and nonsense mutations is most likely because a complete lack of G6PD would be lethal to the developing embryo. In many cases, the same amino acid changes have taken place repeatedly implying that there are a limited number of amino acid changes that can give rise to the phenotype of CNSHA (Mason *et al*., 2007).

III.1. Techniques of diagnosis

The diagnosis of G6PD deficiency is made by a quantitative spectrophotometric analysis or, more commonly, by a rapid fluorescent spot test detecting the generation of NADPH from NADP (Gregg and Prchal, 2000). Infield research, where quick screening of a large number of patients is needed, other tests have been used; however, they require definitive testing to confirm an abnormal result (Iwai *et al*., 2003 ; Jalloh *et al.*, 2004). Tests based on polymerase chain reaction detect specific mutations and are used for population screening, family studies, or prenatal diagnosis (Beutler, 1994).

III.1.1. Biochemical diagnosis

III.1.1.1. Measurement of G6PD activity

G6PD promotes the conversion of its specific substrate glucose-6-phosphate (G6P) to 6-phosphoglucono-d-lactone (6PGL) with a simultaneous reduction of the coenzyme NADP to NADPH. The 6PGL produced undergoes hydrolysis either spontaneously or via lactonase to 6 phosphogluconate (6PG), and is the substrate for the next enzyme in the metabolic pathway, 6-phosphogluconate dehydrogenase (6PGD), which is also present in the red cells, and which also reduces an additional amount of NADP to NADPH (Glock and McLean, 1953).

Since NADPH is produced by both reactions, the only way to measure true G6PD activity is by carrying out assays with two different reaction mixtures: one containing an excess of both G6P and 6PG and the other containing only 6PG. The difference in activity between the first (G6PD + 6PGD) and the second (6PGD) assay gives the true G6PD activity (Glock and McLean, 1953). Although this essay can be regarded as more accurate for certain research purposes, it is not necessary for diagnostic purposes (British Society for Haematology, 2020).

Indeed, for G6PD deficiency, it tends to introduce an error greater than the one it is meant to correct for. The WHO method which measures the overall reaction is satisfactory and is simpler to perform. commercial kits simulate the Glock and McLean method by incorporating maleimide in their reagent to inhibit the G6PD activity. All tests for measuring G6PD activity depend on detecting the rate of reduction of NADP to NADPH and are based on one of the following properties of NADPH (British Society for Haematology, 2020) :

1) Absorption of light at 340 nm.

2) Fluorescence produced by long-wavelength UV light (approximately 340 nm).

3) Ability to decolorize or lead to the precipitation of certain dyes.

III.1.1.2. Fluorescent spot test ("Beutler's" fluorescent spot test)

This test is recommended by the International Council for Standardisation in Haematology (ICSH) (Beutler *et al.,* 1979). The method consists of the following steps :

1) To the reagent mixture that consists of buffered solution of glucose-6-phosphate, NADP, saponin, and GSSG, whole blood is added. G6P is a substrate for G6PD; saponin is used for lysis of red cells; and GSSG oxidizes a small amount of NADPH formed and thus renders the test more sensitive for the detection of mild G6PD deficiency (Mura *et al.,* 2009).

2) A drop (spot) of this mixture is applied to the filter paper and examined under ultraviolet light. The following controls should always be run to test the accuracy of results: positive control (known G6PD-deficient sample) and negative control (normal or non-G6PD deficient sample).

If G6PD is present in the test sample then NADPH is produced from NADP. NADPH fluoresces under ultraviolet light while NADP fails to do so. The presence of fluorescence indicates normal G6PD activity, while the absence of fluorescence indicates G6PD deficiency (< 20% activity). This test is simple, specific, and requires an only small amount of blood. It is used for the diagnosis of G6PD deficiency in individual cases and in population surveys (Fig. 6) (Mura *et al.*, 2009).

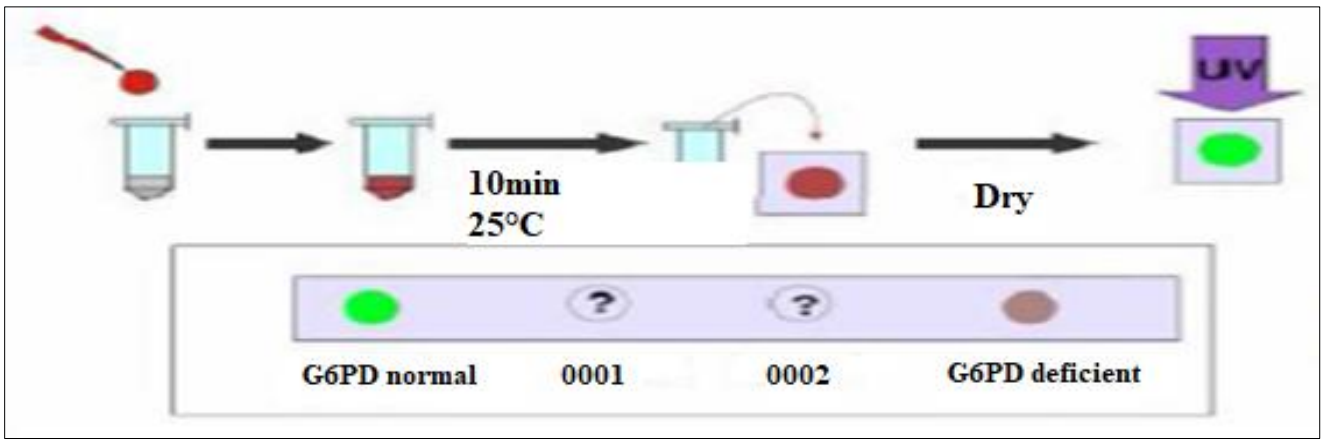

Fig. 6. Beutler's fluorescent spot test (Waal, 2013).

III.1.1.3. Quantitative spectrophotometric technique of enzyme activity

The positivity of the diagnostic test must always be confirmed by measuring the enzymatic activity, a standard technique. The hemolysate is incubated with G6P and a reaction mixture containing NADP. In the presence of NADP, G6P is oxidized to 6-Phosphogluconate by G6PD (Fig.7) (Mura *et al*., 2009).

There is concomitant production of NADPH, H+ which absorbs at 340 nm, unlike NADP. The variation in absorbance as a function of time is proportional to the G6PD activity. In normal red blood cells, the G6PD activity measured at 37°C is 7 to 10 IU/g of hemoglobin (Mura *et al*., 2009).

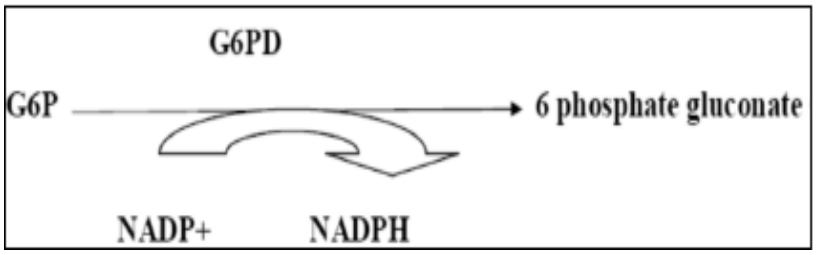

Fig. 7. The production of NADPH by the transformation of G6P into 6-Phosphogluconate by the enzyme G6PD (Waal, 2013).

III.1.1.4. NADP reduction test

It based on the measurement of the fading time of brilliant cresyl blue. Resulting in discoloration which is delayed in subjects deficient in G6PD. It is a colorimetric method (Dembol, 2008). The hemolysate is incubated with a buffered mixture of G6PD and NADP dye. If G6PD exists in the hemolysate, it converts NADP to NADPH. NADPH reduces the dye to a colorless compound. In the presence of a G6PD deficiency, the time taken for the discoloration dye is longer (Kawthalka, 2012).

III.1.1.5. Reduced glutathione stability test

It is done by incubating the blood with acetylphenylhydrazine. In the blood of the normal subject, the level of glutathione reduced does not decrease little, while a significant drop is observed in the blood of subjects with the enzyme deficiency. This test measures the rate of reduced glutathione formation in red blood cells (Girot, 2012).

III.1.2. Haematological diagnosis

V.1.2.1. Blood smear

It makes it possible to study the morphology of the figured elements of the blood and to determine if there is an abnormality (presence/absence, appearance, number) of the blood cells (Girot, 2012).

III.1.2.2. Haemogram or Full Blood Count

It consists of a quantitative and qualitative study of blood cells (leukocytes, red blood cells, and blood platelets) and provides information on the level of reticulocytes. This is the first exam to provide useful information to suspect a hemoglobin abnormality. It is done at a distance from any transfusion (Girot, 2012).

III.1.2.3. Heinz body search

During acute hemolysis, the examination of the blood smear by supravital staining (crystal violet, new methylene blue...) can guide the diagnosis by showing the presence of inconsistent Heinz bodies (Fig.8). Indeed, their presence on the smear is transient because they are quickly sequestered in the spleen. They are also non-specific because they are also found in hemoglobinopathies with unstable hemoglobin and in glutathione reductase deficits (Mura et al., 2009).

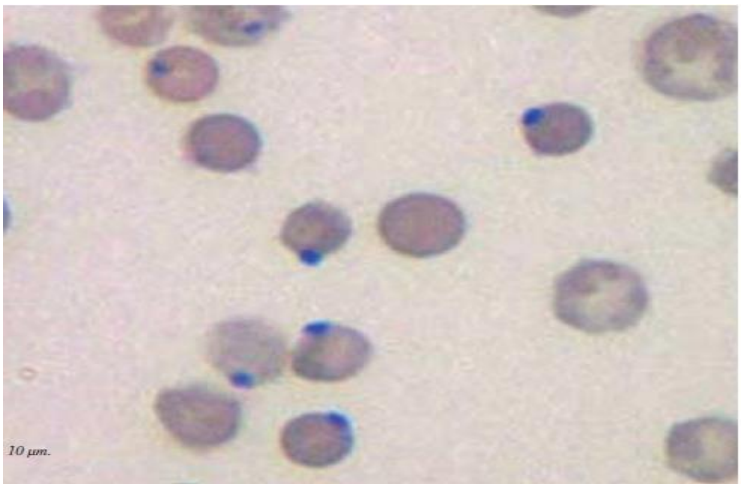

Fig. 8. Heinz body with new methylene blue (x500) (Mura *et al.*, 2009).

III.1.3. Molecular biology diagnosis

Molecular analysis may be useful for population screening, family studies, or parental diagnosis, although this approach is not used routinely. For a correct laboratory practice, the molecular diagnosis of G6PD deficiency should employ two analytical steps:

1) A first screening level, to research the most frequent mutations belonging to a specific geographical area. In this case, a PCR coupled to RFLP represents a rapid valid, and reliable molecular screening approach (Minucci *et al.,* 2009).

2) A second level, based on the whole gene sequencing, finalized to the identification of the less frequent, or novel mutation (Minucci *et al.,* 2009).

DNA based test for the screening of the most frequent mutations in a specific geographical area can be used as an alternative tool to the biochemical assay. The costs for chemicals dedicated to molecular test are comparable to those used for the enzyme assay. In the future, more advance systems should be utilized to improve the efficiency of the molecular assay (Aster, 2007).

3.2. Treatment

The main treatment for G6PD deficiency is the avoidance of oxidative stressors. Rarely, anemia may be severe enough to warrant a blood transfusion. Splenectomy generally is not recommended. Folic acid and iron potentially are useful in hemolysis, although G6PD deficiency usually is asymptomatic and the associated hemolysis usually is short-lived. Antioxidants such as vitamin E and selenium have no proven benefit for the treatment of G6PD deficiency (Beutler, 1994). Research is being done to identify medications that may inhibit oxidative- induced hemolysis of G6PD-deficient red blood cells (Sharma *et al*., 2003).

The 202A/376G G6PD A- allele is considered to be the most common G6PD deficiency allele in sub-Saharan Africa. This allele has two non-synonymous single nucleotide polymorphisms (SNPs) at positions 202 and 376 of the coding sequence (Hirono et Beutler, 1988) :

- G6PD A(+) base substitution A(376) → G with amino acid substitution Asn → Asp.
- G6PD A(-) base substitution G(202) → A with amino acid substitution Val → Met.

IV.1. Distribution of the African form in the Arab countries

In Tunisia (2013), Benmansour et al. have screened 423 patients after hemolysis triggered by fava beans ingestion, neonatal jaundice, or drug hemolysis. Others were asymptomatic but belonged to a family with a history of G6PD deficiency. The determination of enzymatic activity using the spectrophotometric method revealed 293 deficient (143 males and 150 females). The molecular analysis was performed by a combination of PCR-RFLP and DNA sequencing to characterize the mutations causing G6PD deficiency. Fourteen different genotypes have been identified among them the African form, which was present G6PD A- (376A > G ; 202G > A) (46.07%) (Benmansour *et al.,* 2013).

In 2014, a total of 2100 Saudian male subjects were screened; of these, 100 (4.76%) were shown to have G6PD deficiency. Blood samples from these subjects and 100 controls (selected randomly from the 2000 remaining samples) were used for molecular analysis for the presence of the G202A and A376G mutations. On molecular characterization, 6% of those with G6PD deficiency had the A376G mutation and 2% had the G202A mutation, giving an overall frequency of the G6PD A- mutation of 2%. However, this was not statistically significantly different compared with the frequency of this mutation in controls. The findings of this study were limited by the small sample size but it confirmed the existence of African form in the Saudi Arabia population (Alharbi *et al*., 2014).

In 2010, a molecular study had performed on Iraqi male Kurds among 115 G6PD deficient males revealed that had no cases of G6PD A- (Al-Allawi *et al.,* 2010). In 2012, a total of 1810 healthy adult male blood donors were randomly recruited from the national blood transfusion center in Baghdad. They were classified into G6PD deficient and non-deficient individuals based. DNA from deficient individuals was studied using a polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP) for four deficient

molecular variants. G6PD deficiency was detected in 109 of the 1810 screened male individuals (6.0%). Among 101 G6PD deficient males molecularly studied, the African mutation was detected in G6PD A- in two cases (2.0%) (Al-Musawi *et al.,* 2012). Another study was conducted between May 2012 and May 2013. A total of 61 G6PD deficient male individuals from Nineveh province were enrolled in this study. DNA from all enrolled individuals were extracted and analyzed for four deficient molecular variants using a polymerase chain reaction-restriction fragment polymorphism method. The African A- variant (202 G/A) was found in only one (1.64 %) of the deficient individuals and this is comparable to that from Baghdad (Kashmoola *et al*., 2015).

In Jordan (2012), 25 blood samples were collected from patients attending King Abdullah University Hospital and Princess Rahma Teaching Hospital. The G6PD gene was scanned for mutations using a DNA sequencing technique. The results showed 11 variations. Among these was G6PD A- (c.202 G/A + c.376 A/G) with 19% (Al-Sweedan *et al*., 2012).

In 2003, 1080 Kuwaiti males have screened blood donors for G6PD deficiency revealed this condition in 70 (6.5%) individuals. Mutation analysis of all 70 G6PD deficient samples performed by PCR-RFLP and direct sequencing identified the African form (202 G/A) in 14.3% (Samilchuk *et al*., 2003). A study in 2005 used the DNA extract of 82 G6PD deficient Kuwaiti subjects (75 men and 7 women) and screened for gene mutations using polymerase chain reaction/restriction fragment length polymorphism and PCR/single-strand conformation polymorphism followed by direct sequencing. A total of 1209 randomly selected Kuwaiti adult subjects of both sexes were then screened for any characterized mutation (Al-Fadhli *et al*., 2005). The G6PD Mediterranean 563C→T, and 202G→A, 376A→G genotypes were characterized as the most common variants among the G6PD deficient population, representing 0.742 and 0.124 allele frequencies, respectively (Al-Fadhli *et al*., 2005).

Eighty unrelated Palestinian children hospitalized for hemolysis were studied. G6PD activity was determined by quantitative spectrophotometry and G6PD mutations were analyzed by the sequencing of DNA for the results: 65 of 80 children (81%) had G6PD deficiency, accounting for most of the hemolytic disease in this age group. G6PD Mediterranean 563T, African G6PD A- c.202A/c.376G, and G6PD Cairoc. 404C were common with relative allele frequencies of 0.33, 0.26, and 0.18 respectively (Sirdah *et al.,* 2012). The prevalence (28.6%) of G6PD A- c.202A/c.376G among G6PD deficient patients

within the cohort is the third highest after the Arab African countries: Tunisia (63.63%) and Algeria (46%) (Feldman, 2008).

IV.2. Distribution of the African form in the Mediterranean countries

Red cell G6PD deficiency is not rare in Turkey. The frequency of this enzyme deficiency in Turkish males was reported to vary between 0.5-11.4% depending upon geographical areas and/or ethnic groups (Say *et al*., 1965 ; Altay *et al*., 1978). Molecular studies of red cell G6PD enzyme revealed the presence of about 122 mutations which were recently reviewed by Vulliamy et al (Vulliamy *et al.,* 1997). A total of 50 unrelated male subjects with red cell G6PD deficiency were the subjects of this study. The diagnosis of enzyme deficiency was made in the neonatal period in 40 of 50 subjects during screening studies for hyperbilirubinemia. In the remaining 10 subjects, enzyme deficiency was diagnosed at the time of a hemolytic crisis. Genomic DNA was obtained from peripheral blood using standard methods (Oner *et al*., 2000). PCR was used to amplify the portions of the coding region of the G6PD gene as described previously (Poggi *et al*, 1990). The study revealed that the enzyme deficiency was associated with the African type of G6PD deficiency A- (376G/202A) in 2 of 50 subjects (4%) (Oner *et al*., 2000).

In order to explore the nature of G6PD deficiency in Spain, researchers have analyzed the G6PD gene in 11 unrelated spanish G6PD deficient males and their relatives by using the PCR and single-strand conformation polymorphism analysis (PCR-SSCP) combined with a direct PCR-sequencing procedure and PCR-restriction enzyme analysis (RE). Eight different missense mutations have been identified, 6 of which have been reported in previously described G6PD variants. The study revealed that the enzyme deficiency was associated with the African type of G6PD deficiency A- (376G/202A) in 4 of 11 subjects (36 %) (Rovira *et al*., 1995). The substitutions at nucleotide 376 and 202 accounts for 4 out of 11 samples examined, corroborating the high prevalence of G6PD A- among G6PD-deficient Spanish individuals (Rovira *et al*., 1994).

Researchers examined 161 G6PD deficient subjects (130 males and 31 females) originating from different parts of Italy. G6PD activity and molecular characterization were determined in all the subjects analyzed (Di Montemuros *et al*., 1997). Blood samples were collected in acid-citrate-dextrose (ACD) or EDTA after informed consent. G6PD activity was determined in all the subjects according to the WHO and ICSH recommendations (Betke *et*

al., 1967 ; Beutler *et al.*, 1977). Electrophoretic mobility on cellulose acetate gels was assessed in 115 samples according to the method of Rattazzi et al (Rattazzi *et al*., 1967) with minor personal modifications in three different buffer systems (Tris, Borate, Phosphate). Seventy G6PD deficient samples were biochemically characterized in a previous study. The study found the G6PD A- in 4% of the samples analyzed (Di Montemuros *et al.*, 1997).

After the analysis of different studies about G6PD deficiency distribution in Arab and Mediterranean countries, we have concluded that the prevalence of the G6PD African form (G6PD A-) is reported to have a low prevalent in Tunisia (46,07%), Saudi Arabia (2%), Irak (1,64% - 2%), Jordan (19%), Kuwait (14,3%), Palestine (28,6%), Turkey (4%), Spain (36%), Italy (4%).

G6PD deficiency is one of the most prevalent polymorphisms in the world. Its adverse effects include hemolytic anemia and neonatal jaundice but, nonetheless, the gene frequencies of the various types of G6PD deficiency have risen to such high levels that it has been estimated that there are 400 million G6PD deficient persons in the world.

The variety of different mutations that cause G6PD deficiency is been greatly clarified in the past decade through studies of the molecular biology of the deficiency.
Molecular testing has been important in identifying the different variants which have clinical significance. Aside from its interest as a major sexlinked polymorphic marker, G6PD has served as an important tool as a tracer in various biologic studies.

The G6PD A- deficient genotype was not relatively common among the diverse Arab and Mediterranean populations included in that analyse. This represents a significant risk for adverse haemolytic events after treatment with drug therapies that have the potential to induce oxidative stress. G6PD genotyping and phenotyping should therefore be requisite in clinical trials evaluating the safety and efficacy of such drugs.

In general, distribution prevalence of G6PD A- is low in Arabic and Mediterranean countries, it has shown a low proportion in Arabia Saudia, Kuwait, Jordan, Irak, Palestine, Turkey, Spain, and Italy, although G6PD Mediterranean is described as the predominant variant. A unique finding in this study was the high prevalence of the G6PD A- in Tunisia (46,07%).

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Presented by: Lamrani Abir, Bounab Meriem	**Theme:** Glucose-6-phosphate dehydrogenase : distribution of the African form in the Arab and Mediterranean countries.

Summary:

Glucose-6-phosphate dehydrogenase (G6PD) is a critical enzyme for protecting red blood cells from oxidative damage. G6PD deficiency is an X-linked recessive disorder caused by mutations in the G6PD gene and affects nearly 400 million people worldwide. Among G6PD deficient individuals, it is common to experience the premature breakdown of red blood cells in the face of oxidative stress.

It has been found in the literature that the African variants of G6PD A- is more common in the Arab countries than in the Mediterranean's, but its percentage is low compared to other forms of G6PD deficiency.

Keywords: G6PD deficiency, G6PD A-, Arab and Mediterranean countries.

Résumé:

La glucose-6-phosphate déshydrogénase (G6PD) est une enzyme essentielle pour protéger les globules rouges des dommages oxydatifs. Le déficit en G6PD est un trouble récessif lié au X causé par des mutations du gène G6PD et affecte près de 400 millions de personnes dans le monde. .

Il a été constaté dans la littérature que la forme africaine G6PD A- est plus fréquente dans les pays arabes que dans les pays méditerranéens, mais son pourcentage est faible par rapport aux autres formes de carence en G6PD.

Mots clés: déficit en G6PD, G6PD A-, pays arabes et méditerranéens.

ملخص :

إنزيم نازعة هيدروجين الغلوكوز 6 فوسفات هو إنزيم أساسي لحماية خلايا الدم الحمراء من الأكسدة ونقصه ناتج عن طفرات في جين متنحي مرتبط بالكروموزوم X ويأثر على ما يقرب من 400 مليون شخص في جميع أنحاء العالم.

من خلال بحثنا في دراسات سابقة حول انتشار النوع الافريقي (G6PD A-) في الدول العربية ودول البحر الابيض المتوسط وجدنا أنه الأكثر شيوعا في الدول العربية منه في دول البحر الابيض المتوسط، لكن نسبته قليلة مقارنة بالانماط الأخرى الموجودة .

الكلمات المفتاحية : عوز G6PD، النمط الافريقي، الدول العربية والمتوسطية.