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A Brief Narrative Review of Glucose 6-phosphate Dehydrogenase Deficiency in Children and Its Genetic Variants

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Abstract: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most frequent enzyme deficiency which leading to substantial morbidity and mortality in children. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme present in cytoplasm, coded by X-linked gene. G6PD catalyzes the initial step in hexose monophosphate shunt, which is a rate limiting reaction. This shunt helps in producing NADPH (Nicotinamide adenine dinucleotide phosphate), a reducing equivalent that helps glutathione reductase to regenerate reduced glutathione (GSH). Due to its crucial role in defence against oxidizing species, it is perceived that any mutation in G6PD encoding gene can affect enzyme's structural and functional integrity leading to malfunctioning of enzyme. G6PD deficiency is a highly prevalent genetic disorder which is transmitted as X–linked recessive pattern. Nearly 210 variants have been reported all over the world. In India, G6PD deficiency is a significant public health problem. It is an important cause for drug induced hemolysis in children. G6PD gene variants are often named as per the geographical area where they were first discovered. In India Mediterranean, Orissa, Jammu, Andhra Pradesh, Kalyan-Kerala and G6PD West Bengal are frequently seen. G6PD Mediterranean leads to severe deficiency when compared to others. Worldwide highest preponderance of g6PD deficiency is seen in African sub Saharan region and Arabian Peninsula.

Keywords: Glucose-6-phosphate Dehydrogenase, G6PD Gene, Mutation, Mediterranean, X-linked

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the commonest significant genetic enzyme defect, affecting nearly 400 million people across the globe. In India, annually, around 390,000 have G6PD deficiency leading to substantial morbidity and mortality in childhood [1]. Currently, in urban areas it constitutes the fourth most important cause (9.2%) of neonatal deaths [1]. Reasons for this may be the consanguineous marriages which are still still frequent in many regions of India and increased awareness as well as detection of these disorders which previously were undetected. The prevalence of this disorder is highest in tropical regions like Africa, the Middle East, Asian subcontinent (tropical and subtropical areas), Papua New Guinea and few parts of the Mediterranean.

2. Glucose-6-phosphate Dehydrogenase Enzyme

2.1. Function of Glucose-6-phosphate Dehydrogenase Enzyme

G6PD is a vital enzyme present in all cells that catalyzes the initial step of the hexose-monophosphate pathway (HMP) producing NADPH (Nicotinamide adenine dinucleotide phosphate). NADPH is required by cells for numerous reactions in biosynthetic pathways. It also maintains the redox potential that protects the erythrocyte membranes from any oxidative stress and injury [2]. The most common clinical manifestations due to G6PD deficiency include neonatal jaundice and acute hemolytic anemia [1]. Various drugs, like primaquine, infections or foods (fava beans) can precipitate this phenomenon. The hemolysis results due to the inability of G6PD-deficient erythrocytes to tolerate the oxidative damage produced either directly or indirectly by these aggravating agents. The G6PD enzyme has around 200 genetic variants [3]. The coding gene is found on the X chromosome and around 186 genetic variants have been described, which may induce functional enzyme deficiency [3]. Several G6PD gene variants are named according to the areas/ regions from where they were first indentified; for instance - Canton, Mahidol, Kaiping and Viangchang (more common in Asian populations) and G6PD Kalyan-Kerala, Mediterranean, Coimbra, Orissa, G6PD* Andhra Pradesh, G6PD*Cutch, G6PD* Jammu, G6PD* Kerala-Kalyan, G6PD*Porbandar, G6PD*Chatham, G6PD* Insuli, G6PD*Coimbra, and G6PD*West Bengal are other variants seen in India [3-5]. In General the Indo-European and Austro-Asiatic language groups were found to have higher G6PD deficiency than the Dravidian language speaking groups.

2.2. Structure of Glucose 6-phosphate Dehydrogenase Enzyme

Glucose 6- phosphate dehydrogenase enzyme is a member of oxido-reductase family. There exists equilibrium between dimeric to tetrameric form in active state of G6PD. At high ionic strength and alkaline pH there occurs a shift of equilibrium towards dimeric form, and reversibly at acidic pH and low ionic strength this equilibrium shifts towards tetrameric form. Binding of $NADP^+$ (Nicotinamide adenine dinucleotide phosphate) favours tetramerization of two G6PD dimers. Adenine of $NADP^+$ lies between Arg487 and Tyr 503 and nicotinamide is sandwiched between Trp509 and Tyr 401. Also, 2' phosphate makes hydrogen bonds with Arg487, Arg357, Lys238 and Lys 366. Role of Arg487 is distinctive of $NADP^+$ binding sites in several enzymes [6-8].

2.3. Enzymology

In HMP pathway glucose -6- phosphate is being oxidized to 6- phospho- glucono- δ- lactone, along with $NADP^+$ being reduced to NAPDH. NADPH serves as a reducing equivalent in many anabolic processes in the body. As D-Ribose 5-phosphate is the end product of HMP pathway, the the overall reaction results in:

$$2NADP^+ + \text{Glucose 6-phosphate} + H_2O \text{ ---------- } 2NADPH + 2H^+ + \text{Ribose 5-phosphate} + CO_2$$

Besides the above-mentioned reaction, there are various metabolic processes in the cell that produce NADPH. This meets cell's requirement of reducing equivalents. However, during the course of erythrocyte maturation, there is loss of mitochondria, making HMP pathway as the only provider of NADPH. NADPH is an essential reductant in anabolic processes, also playing a protective role against oxidative damage by reactive oxygen species like H_2O_2, O^{2-}. Various metabolic pathways and by the action of some drugs (e.g.: primaquine; an anti- malarial drug), highly reactive oxidants are produced. Also, some natural products such as divicine, a toxic component of fava beans can lead to oxidant damage resulting in favism.

NADPH regenerates reduced glutathione (GSH), as it provides hydrogen to oxidized glutathione (GSSG) in a reaction catalyzed by glutathione reductase. This reduced glutathione is used by glutathione peroxidase which converts hydrogen peroxide into water molecule. Hence it serves as an antioxidant regenerator in reactive oxygen species (ROS) scavenging mechanism [7, 8].

3. Epidemiology of G6PD Deficiency

G6PD deficiency is the fifth common congenital defect across the world, and the most common erythrocyte enzymopathy. It affects around 400 million people globally with a worldwide prevalence of 4.9% [9, 10]. It is highly prevalent in tropical (Asia & Africa), subtropical regions alongside Mediterranean and Middle East countries [10]. The prevalence of G6PD deficiency is highest in Arabian Peninsula and sub- Saharan Africa [11]. As of now 210 G6PD mutations have been outlined. The most common being single nucleotide substitution (missense variants), followed by two or more substitutions, deletions, and intron affecting mutations [10, 12].

Role of G6PD deficiency has been elucidated in protection against Malaria infection due to hindered antioxidant defence in ring-staged parasitized erythrocytes *Plasmodium falciparum* and *Plasmodium vivax* are important plasmodium species causing malaria posing threat to 3.3 billion people globally [11-18]. As the gene from which G6PD is being translated is highly polymorphic with around 210 variants. In males phenotypic expression of this gene is variable. While in females, who are heterozygous the manifestations are based on the inactivation of X-chromosome. Countries having high malaria transmission have good correlation with prevalence of G6PD deficiency [11]. On the contrary, recently it has been reported that G6PD deficient individuals are more prone for acquiring SARS-CoV-2 infection.

4. G6PD Enzyme Activity Classification

According to WHO (World Health Organisation) Malaria Policy Advisory Committee meeting report (October 2019), G6PD enzyme activity was classified as shown in Table 1. 25% or less activity of G6PD enzyme is often associated with clinical manifestations of hemolysis in children. Also, these children are at risk of drug induced hemolysis.

Previously, Yoshida et al.'s proposed classification of G6PD, in the year 1971, also known as the "WHO Classification " as shown in Table 2 [19]. Although very old, it still holds relevance till today. Class I mutations are usually shown to involve the exons 6 (attaches to the enzyme substrate) 8, (present at dimer interface), 10, and 13 (NADP+ structural site) [10]. It is not essential that every G6PD variant belonging to class II or III need to be polymorphic, although it is probable. Table 3 enumerates predominant G6PD variants seen in India.

Table 1. *G6PD enzyme activity According to WHO (World Health Organisation) Malaria Policy Advisory Committee meeting report (October 2019) [13].*

G6PD activity	Indicator sign
25% or less activity	(-)
25-65% activity	(+/-)
65-150% normal activity	(+)
>150% activity	(++)

Table 2. *Classification of degree of deficiency and clinical manifestations based on G6PD activity (Class I – V) [19].*

Degree of Enzyme deficiency	Class	Residual G6PD activity (% of Normal) in hemizygous males	Clinical finding
Severe	I	<10%	Chronic non-spherocytic hemolytic anemia (CNSHA)
Severe	II	<30%	None in steady state
Moderate to mild	III	>30%	Intermittent acute hemolysis
Very mild or no deficiency	IV	>85%	None
> twice of normal	No deficiency but increased enzyme activity		None

Cutoff is indicated as 30%, because all G6PD variants in class II and III described so far have a residual activity of less than 30%.

Table 3. *Most frequent G6PD variants in India and their genomic charachteristics [11, 42, 43].*

Variant name	cDNA nucleotide substitution	Genomic nucleotide substitution	Amino acid substitution	Clinical effects (WHO class)
Mediterranean	563 C-T	11734T	188 Ser-Phen	Class 2
Kalyan kerala	949 G-A	13109A	317 Glu-Lys	Class 3
Orissa	131 C-G	9988G	44 Ala-Gly	Class 3
Coimbra Shunde	592 C-T	11763T	198 Arg-Cys	Class 3
Jammu	871 G-A	13031A	291 val-Met	Class 3
Ludhiana	929 G-A	13089A	310 Gly-Glu	Class 3
Guadalajara	1159 C-->T	13458T	387 Arg-->Cys	Class 3
Chatham	1003 G-A	13163A	335 Ala-Thr	Class 3

5. Clinical Impacts of G6PD Deficiency in Children and Neonates

Genetic abnormalities in G6PD deficiency may remain largely asymptomatic throughout the life, due to which people often remain unaware of being G6PD deficient. When exposed to oxidant stress like exposure to drugs (antimalarial), infection or some natural plant products like fava beans, hemolysis can occur in these individuals [20]. However, it can manifest with chronic non spherocytic hemolytic anemia, neonatal jaundice, favism, acute hemolytic anemia (AHA) induced either by drug or by infection in children [21, 22].

5.1. Drug or Infection Induced Acute Hemolytic Anemia (AHA)

It has been observed that people who are sensitive to primaquine are also sensitive towards other hemolysis triggering drugs [22]. During hemolysis of erythrocytes, hemoglobin being a globular protein undergoes structural denaturation, and precipitates as Heinz bodies. G6PD deficient patients, can be affected by wide range of external agents leading to acute hemolytic anemia [23]. Drug triggered AHA depends upon the dose of administration [20]. There are several familial or sporadic factors which predispose G6PD deficient individuals susceptible to AHA after drug administration. These factors include erythrocyte metabolic function, characteristics of G6PD enzyme defect, genetic manipulation of pharmacokinetics and pharmacodynamics (dose, absorption, metabolism and excretion of drug). Similarly, other triggers consist of ongoing oxygen free radicle generation, hemoglobin concentration and old to new erythrocyte ratio [20, 21]. Infection with Hepatitis A, and B, cytomegalovirus, other bacterial infections, can also induce AHA. Most probable reason for AHA could be generation of oxidizing species by leucocytes, transmitting oxidative stress in erythrocytes [2, 21].

5.2. Favism

Favism term is used to describe fava bean induced AHA, first reported in early twenties. Mediterranean G6PD

variant is most frequently associated with favism [21]. Children below 5 years of age are most often affected by favism which can be fatal [23]. It is not necessary that all people who are G6PD deficient may encounter hemolysis after intake of fava beans. Glycosides like iso-uramil, divicine are among some hemolysis triggering compounds of fava beans. These agents accelerate HMP shunt, in turn promoting hemolysis [22]. After 24 hours of ingestion of fava beans, acute hemolytic anemia manifests. Erythrocytes undergo morphological changes due to oxidative damage triggered by fava beans [21].

5.3. Neonatal Hyperbilirubinemia (NNH)

NNH is often a serious clinical complication of G6PD deficiency, which manifests 2-3 days after birth. G6PD Mediterranean, canton variants often results NNH. Thus, screening neonates for G6PD deficiency is crucial for prevention of consequences of NNH [22].

5.4. Chronic Non-Spherocytic Hemolytic Anemia (CNSHA)

CNSHA stems up from most severely affected class 1 G6PD variants [21]. Low enzyme activity in these mutants leads to reduced NADPH production, hindered free radical scavenging and hemolysis. Exon 10 encodes for structural NADP+ and dimer interface of native glucose 6- phosphate dehydrogenase is essential for structural and functional integrity of enzyme, which is affected by nearly 95 class 1 mutations [2]. Individuals with neonatal jaundice and AHA are more susceptible to CNSHA requiring life long blood transfusions [23, 24].

5.5. G6PD and SARS-CoV-2

Recently it has been reported that G6PD deficiency poses as a predisposing factor for SARS-CoV-2. For generating an adequate immune response G6PD is essential. Both G6PD deficiency and SARS-CoV-2 affects the anti oxidant system through the similar pathways rendering G6PD deficient patients easy prey of COVID-19. The G6PD deficiency cohort had an evolutionary antimalarial advantage but they had disadvantage against SARS-CoV-2 [25-28].

6. Qualitative and Quantitative Detection Methods for G6PD Enzyme Activity

6.1. Fluorescent Spot Test (FST)

FST detects G6PD enzyme activity by measuring NAPDH synthesis, which is coupled to fluorophore. In Beutler's qualitative FST G6P substrate (non-fluorescent) and $NADP^+$ were converted into 6-phosphogluconate (fluorescent) and NADPH. For visualization of fluorescence spots UV illuminator is used with 365 nm wavelength. Fluorescence intensity is proportional to G6PD activity [29]. G6PD activity is estimated by the emission of fluorescence in a given time period. In normal G6PD activity there occurs moderate to strong fluorescence occurs by 5 minutes and strong fluorescence by 10 minutes. In deficient activity there occurs no fluorescence in 5 and 10 minutes. In a study done by Sunhee Hwang et al, they have coupled NAPDH production with diaphorase converting resazurin to fluorescent resorufin with excitation wavelength of 565 nm and emission wavelength of 590 nm. FST is reliable for G6PD activity estimation in hemizygous male and homozygous female but not for heterozygous female (as 50% of erythrocytes have fluorescence, masking the deficiency). As it is inexpensive and has easy field application most of the screening tests for G6PD deficiency are based on fluorescent spot test.

6.2. G6PD Rapid Diagnostic Test (RDT)

RDT is a qualitative tool designed on the principle of converting transparent nitro blue tetrazolium dye to purple color reduced formazan. Appearance of purple color indicates normal G6PD activity, and its absence indicates G6PD deficiency [29]. RDT provides results within 5-10 min and have medium to high efficacy. In malaria-endemic countries, G6PD RDT assay has high sensitivity for diagnosing G6PD deficiency in people having less than 30% of normal G6PD activity. RDT can identify severely deficient individuals but has less diagnostic ability in mild G6PD deficiency [30-32].

6.3. Spectrophotometric Assay

It is a quantitative method to estimate G6PD activity. For children above 3 months of age, reference interval for G6PD activity is 3.8 to 5.9 IU/g Hb. G6PD: GR (Glutathione reductase) activity ratio is shown to be 0.9 to 1.31. Changes in absorbance are measured at 340 nm and 540 nm for G6PD activity and hemoglobin concentration respectively. Children with both G6PD activity and G6PD: GR activity ratio less than standard reference threshold are considered to be G6PD deficient. This has 11% sensitivity and 99% specificity for detecting heterozygous females with G6PD deficiency [33]. Spectrophotometric assay has been a gold standard method for detecting the total enzymatic activity.

6.4. Flow Cytometric Analysis

As FST and RDT are non-reliable for G6PD activity detection in heterozygous females, cytochemical analysis is used in them. This detects G6PD activity of individual RBCs [33-35]. Brewer developed Methemoglobin reduction test (MRT) which functions on the principle of reduction of oxidized Methemoglobin to normal hemoglobin by the erythrocytes [33]. Another method explores G6PD reducing ability by analyzing the reduction of water soluble [3-(4, 5-Dimethylthiazolyl2)-2, 5-Diphenyltetrazolium Bromide] (MTT) into water insoluble tetrazolium salts. G6PD-deficient erythrocytes remain unstained, whereas erythrocytes having normal G6PD activity appear with purple granules. Grading of cells which are puple stained is used as a measure of intracellular granularity, which corresponds to intracellular G6PD activity. Subjects with acute malaria and with different

parasite load, erythrocyte dynamics can be studied by flow cytometric assay [33-35].

6.5. Genotyping

Polymerase chain reaction (PCR) along with restriction fragment length polymorphism (RFLP) is used to diagnose and charachterize G6PD gene mutations. This compares the children's gene sequences to a standard reference sequence [35]. Allele specific analysis (ASA) provides genotype status of G6PD deficient individuals rapidly, which requires prior knowledge of that particular G6PD variant or single nucleotide peptide (SNP) [35, 36].

6.6. Next Generation Sequencing (NGS)

G6PD mutation analysis by NGS is an effective modality for genetic screening and diagnosing new mutation with the aid of whole exome sequencing [36-43]. This can also provide insights into personalized treatment. For the detection of single nucleotide variants in NGS data, wide variety of software tools are available. As search for new variants in G6PD gene is ongoing, NGS can play a promising role in identifying new SNPs and point-mutations in G6PD in both cost effective and time effective manner.

7. Conclusion

Amongst the mutations causing G6PD deficiency, point mutations are the most frequent. Other infrequent mechanisms include intron mutations, deletions and multiple mutations. RDT provides quick qualitative estimation of G6PD activity but unreliable in heterozygous female with false negatives. The evolutionary antimalarial benfit of the G6PD deficiency cohort can be an unfavourable entity against SARS-CoV-2. Early detection of this enzymopathy and avoiding exposure to external agents causing hemolytic episodes is the only solution and preventive cure for this entity. Screening all the neonates for G6PD deficiency at birth by augmenting the neonatal screening program is required in India, as G6PD deficiency is an important public health problem. G6PD screening is of importance in treating children with SARS–CoV-19 infection. G6PD deficiency is more prevalent in North India & western part of India compared to southern part.

Declaration of Competing Interest

All authors declare that they do not have any conflict of interest.

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