Molecular Characterization of the Most Common Mutations Causing G6PD Deficiency (Mediterranean and African Mutations) among Egyptian Children

Thesis

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Presented By

Marwa Salah El-Sherif

M.B.B.Ch., M.Sc. Pediatrics
Faculty of Medicine – Ain Shams University

Under Supervision of

Prof. Mohsen Saleh El Alfy
Head Professor of Pediatrics
Faculty of Medicine, Ain Shams University

Prof. Nancy Abd El Aziz Soliman
Professor of Pediatrics
Faculty of Medicine, Ain Shams University

Dr. Amira Abd El Moneam Adly
Assistant Professor of Pediatrics
Faculty of Medicine – Ain Shams University

Dr. Marwa Talaat El-deeb
Lecturer of Pediatrics
Faculty of Medicine – Ain Shams University

Dr. Tarek Mostafa Kamal
Assistant Professor of Pediatrics Genetics
Faculty of Medicine – Ain Shams University

Faculty of Medicine
Ain Shams University
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Marwa El-Sherif

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ABSTRACT

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect. It is considered a health problem, especially in Asia, Middle East and Mediterranean countries with many biochemical and clinical phenotypes. The effective management of G6PD deficiency is to prevent haemolysis by avoiding oxidative stress with fava beans is the commonest. No data is available about the effect of non-fava beans diet in those patients. Molecular characterization of G6PD deficiency variants is essential, since the biochemical characterization has lost its significance due to the individual variability.

Aim: to determine the frequency of the common mutations causing G6PD deficiency in Egyptian children, as well as making genotype-phenotype correlation for the identified mutations affecting G6PD gene on Xq28. Also to investigate the challenge of non-fava beans diet on occurrence of hemolysis.

Patients and Methods: A prospective study involved quantitative analyses for enzymatic activity with molecular typing were performed on 108 G6PD-deficient children. A polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) technique was used to detect the G6PD enzyme mutation with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Patient's medical records were reviewed as regard age at diagnosis, demographic data, the offending agent which precipitated the first attack, history of blood transfusion and G6PD level at diagnosis. Challenging of patients with haemoglobin level $\geq$ 12 gm/dl with intake of non- fava beans diet with monitoring of those patients by complete blood count, reticulocytic count markers of hemolysis.

Results: The G6PD Genotypes detected were; Mediterranean variant mutation in 53% (from which 54.7% had G6PD 1311T silent polymorphism), Cairo mutation in 13% and African mutation in 16% of cases. Chatham mutation in 4%, Santmaria in 1% and Asahi mutation in 1% of cases and these 3 molecular defects were first to be described in Egyptian G6PD deficiency. Of the studied patients 83 % were symptomatic; 64 % demonstrated acute haemolytic crisis with necessity of blood transfusion induced mainly by ingestion of fava beans and 61 % had

history of neonatal jaundice. Acute haemolytic anemia was found in 79% of Mediterranean variant, 56% of African variant, 61.5% of Cairo variant, 50% of Chatham variant, although none of the patients with santmaria and Asahi variants had developed haemolytic crisis. The G6PD enzyme level was significantly lowered in Mediterranean and African mutation compared to other mutations and was not correlated with disease severity. On follow up of patients after ingestion of legumes rather than fava beans taken in small amount (5-20 gm) per day for 3 successive days no attacks of hemolysis were developed as indicated by colour of urine, CBC, reticulocytic count and markers of hemolysis (indirect bilirubin, LDH).

Conclusion: G6PD deficiency Mediterranean mutation is the most common mutation among Egyptian children with G6PD deficiency followed by African and Cairo mutation. This is the first report of G6PD Santamaria, chatham and Asahi among our Egyptian population. Ingestion of small amount of legumes rather than fava beans was not associated with haemolysis in G6PD deficient children.

Introduction

Glucose 6 phosphate dehydrogenase is the most common human enzyme defect being present in more than 400 million people worldwide ***(Cappellini, 2008)***.

The distribution of glucose 6 phosphate dehydrogenase deficiency among the different ethnic groups varied widely ranging from 1% for Egyptians to 11.5% for Iranians ***(Usanga et al., 2000)***.

G6PD catalyses the first reaction in the pentose phosphate pathway. The pentose phosphate pathway provides reducing power in the form of NADPH by the action of glucose 6 phosphate dehydrogenase which is crucial to the protection of cells from oxidative stress. G6PD is also necessary to regenerate the reduced form of glutathione that is produced with one molecule of NADPH ***(Tsai et al., 1998)***.

G6PD deficiency is an X-linked hereditary genetic defect due to mutation in the G6PD gene which causes functional variants with many biochemical and clinical penotypes ***(Cappellini, 2008)***. The G6PD gene is located in the q28 region of the X chromosome and it consists of 13 exons and 12 introns distributed over approximately 20 kb of genomic DNA. The exons range in size from 38 bp (exon 3) to 695 bp (exon 13).

The coding region is in exons 2-13, while exon 1, the first 8 bp of exon 2 and all out the first 88 bp of exon 13 form the untranslated portions of G6PD mRNA ***(Poggi et al., 1990)***.

The illness generally manifests as acute hemolysis which usually arises when red blood cells undergo oxidative stress, G6PD deficiency usually presents as drug induced or infection induced acute hemolytic anemia, favism, neonatal jaundice, or chronic non-sherocytic hemolytic anemia ***(Cocco et al., 1998)***.

About 140 mutations have been described; most are single base changes, leading to amino acid substitution ***(Cappellini, 2008)***.

Molecular characterization of G6PD deficiency variants is essential, especially since the biochemical characterization has lost its significance due to the individual variability as a result cases may be misdiagnosed ***(Arnout et al., 2010)***.

However, even among males, there is marked variability. The responses of different patients with the same mutation to a single drug dose may vary widely and it has been suggested that the acetylator status of the G6PD deficient patients may modulate the response ***(Beutler, 2001)***. Factors that affect individual variability to severity of drug induced oxidative hemolysis are precise nature of enzyme defect, age, dose effect of drug on enzymatic activity ***(Cappellini, 2008)***. The response to the ingestion of fava beans is particularly variable. G6PD

deficiency seems to be a necessary, but not sufficient factor for favism to occur ***(Beutler, 2001)***.

The Mediterranean and A G6PD variants are particularly prevalent in Africa and Southern Europe ***(Joly et al., 2010)***. G6PD A probably reflects gene flow from Africa ***(Calahro et al., 1993)***.

G6PD Mediterranean mutation is one of the most common mutations causing G6PD deficiency among Egyptian children with G6PD deficiencies ***(Arnout et al., 2011)***. The mutation that causes G6PD Mediterranean may have occurred independently in Asia and in Europe (Beutler and Kuhl, 1990).

The Mediterranean and African variants generally produce a more severe clinical phenotype which is not, however, correlated to the enzymatic activity level. Other mechanisms must exist which offer protection from the oxidative stresses which certainly play a role in the clinical expression of G6PD deficiency (Pietrapertosa et al., 2001).

High frequencies of G6PD deficiency have been reported in most countries in the region. G6PD Mediterranean is the highest documented molecular variant found (188 Ser->Phe) G6PD Mediterranean is also reported to be the most frequently detected variants among individuals with G6PD deficiency in the Middle East and Gulf area. Al-Allawi et al. (2010) screened DNA sequentially for five G6PD deficient mutations including

G6PD Mediterranean (563 C→T), G6PD Chatham (1003 G→A), G6PD Cosenza (1376 G→C), G6PD Aureus (143 T→C), G6PD A-(202 G→A) and for silent (1311 C→T) mutation.

Aim of the Work

The present study aims at:

1- Genotyping of the most common mutations causing G6PD deficiency in Egyptian children.

2- Making genotype-phenotype correlation for the identified types of mutations affecting G6PD gene on Xq28.

3- Trying to make correlation with the different external factors that may increase the susceptibility to different types of mutations affecting G6PD gene.

4- Trying to make a demographic distribution for the differently detected G6PD gene mutations in light of the high admixture of the Egyptian population.