Frequency of Mediterranean mutation among a group of Saudi G6PD patients in Western region-Jeddah

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SUMMARY

Glucose-6-phosphate dehydrogenase deficiency (G6PD), a common human enzymatic defects characterized by extreme molecular and biochemical heterogeneity is found to have a variable frequency in different regions. The molecular basis of polymorphic variants in Saudi Arabia have yet to be fully addressed to. Accordingly, a study was designed to determine the frequency of G6PD gene mutations in G6PD deficient cases. From forty-seven unrelated G6PD-deficient subjects, DNA was extracted individually from peripheral blood samples and exons 6 and 7 of the G6PD gene were amplified by PCR. Mutation analysis was carried out by using conformation sensitive gel electrophoresis (CSGE), followed by direct DNA sequencing. The results showed definite altered CSGE patterns. Two mutations were resolved in exon 6 of G6PD gene; Mediterranean mutation and Sibari mutation, not previously reported so far; while no mutation was detected in exon 7. The frequency of exons 6 mutations responsible for G6PD deficiency (Mediterranean type) is reported for the first time from this region, with a figure of 50.1%. The absence of other mutations in exon 7 causing G6PD deficiency points to the low genetic diversity in the studied population.

INTRODUCTION

The G6PD deficiency afflicting an estimated 400 million individuals worldwide is asymptomatic but acute hemolysis may occur in certain conditions. Rarely, it may cause chronic non-spherocytic hemolytic anemia (Beutler, 1991; Brown and Boon, 1968; Tan, 1981; Fok, Lau & Hui, 1986). The common clinical manifestations of G6PD deficiency are jaundice and acute hemolytic anemia triggered by certain drugs,

infections or ingestion of fava beans (Luzzatto, Mehta & Vulliamy, 2000).

The recent advances in techniques have allowed an accurate molecular characterization of the G6PD gene and a number of variants have been identified (Beutler, 1991). The abnormalities for G6PD deficiency have also extended to ethnic groups in Asia, China (Chang *et al.*, 1992; Tang *et al.*, 1992; Chiu *et al.*, 1993; Lo *et al.*, 1994; Xu *et al.*, 1995; Ainoon *et al.*, 1999), Taiwan (Tang *et al.*, 1995), Indonesia (Soemantri *et al.*, 1995), and Southeast Asia (Iwai *et al.*, 2001). There are reports for population-based studies on G6PD heterogeneity in Middle East. Also (Al-Ali *et al.*, 2002; Samilchuk *et al.*, 2003). However, in spite of initial indications of G6PD deficiency in Saudi Arabia, not much has been performed so far for this region (el-Hazmi & Warsy, 1989). A report on molecular study of G6PD mutation, did appear from Eastern province of Saudi Arabia (Al-Ali *et al.*, 2002) while, no such estimate has been performed for western province. This prompted us to undertake the molecular characterization of G6PD gene in ethnic Arab Saudi population of this region. We determine here the frequency of molecular abnormalities in referred G6PD-deficient adults using CSGE-PCR and direct DNA sequencing techniques.

MATERIALS AND METHODS

Subjects

The study was conducted on 862 individuals (431 women and 431 men; ranging 18–42 years) reporting Maternity and Children Hospital, Jeddah for premarital checkups. The 5 ml of peripheral blood was collected in EDTA from each individual to perform routine hematologic investigations comprising blood (CBC) and reticulocyte count, hemoglobin electrophoresis and quantitative analysis in G6PD cases.

G6PD activity assay

RBC-based G6PD activity was measured as per norms (using UDI kit, Dammam, Saudi Arabia) and the enzyme activity was determined in a cell lysate by plate-reader spectrophotometer (ThermoMax Microplate Reader, PerkinElmer Life and Analytical Sciences Inc., Downers Grove, IL, USA). The rate of increase in absorbance was measured at 340 nm from the conversion of NADP+ to NADPH by G6PD.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using Qiagen DNA extraction kit (Abdulla Fouad Holding Company, Damman, Saudi Arabia). The procedure followed laid out norms for DNA extraction.

Amplification of exons 6 and 7

The entire coding sequence of intron/exon boundaries corresponding to exon 6 and 7 of G6PD gene was amplified by oligonucleotide primers (Table 1). The primers were site specific for Mediterranean and African type variant in respective exons. All PCR reactions were comprised of: 500 ng of genomic DNA, 16.6 mM-$(NH4)_2$ SO_4, 67 mM Tris–HCL (PH 8.0), 10 mM β-mercaptoethanol, 100 μg bovine serum albumin (BSA), 300 ng of forward and reverse primer, 200 μM dNTPs, 1.5 mM MgCL 2, and 1 U *Taq* DNA polymerase in a final volume of 50 μl reaction mix. Samples were initially denatured at 94 °C for 5 min and subsequently amplified using 35 cycles of denaturation at 94 °C for 1 min, followed by annealing at 55 °C for 30 s and extension at 72 °C for another 30 s. The 5 μl of each PCR product was loaded onto 5% polyacrylamide gel for the amplification.

Mutation detection and direct DNA sequencing

Conformation sensitive gel electrophoresis (CSGE) was used to screen for point mutation as of Williams *et al.* (1998). The patient's PCR products were mixed

Table 1. Oligonucleotide sequences used to amplify exons 6 and 7 with PCR product sizes

Exon #	Primer sequences	PCR product (bp)
Exon 6	FW 5′-GCAGCTCTGATCCTCATCCC-3′ RV 5′-GTGAGG GGTCACCCTTGTCT-3′	342
Exon 7	FW 5′-CGAATTCCTCCAGAACTCAGA-3′ RV 5′-GAGGAGCTCCCCCAAGATAG-3′	318

with normal PCR and denatured by heating to 95 °C for 5 min and then incubated at 65 °C for 30 min for heteroduplex formation. The PCR products were electrophorezed on 10% polyacrylamide gel consisting of; 99 : 1 acrylamide (BDH): bis-acrolypiperazine (BAP; Fluka, Buchs SG, Switzerland), 10% ethylene glycol (Sigma-Aldrich, Al-Khobar, Saudi Arabia), 15% formamide (Sigma-Aldrich) and 0.5′ TTE buffer (1′ TTE = 89 mM Tris, 28.5 mM taurine, 0.2 mM EDTA).

Samples displaying abnormal CSGE pattern were compared with pattern from normal individual and subjected to direct sequence (ABI PRISM 377 DNA Sequencer; AME Bioscience Ltd., Bedfordshire, UK). Purification of PCR products was carried out by GFX column System Gel Band Purification Kit (Life Technologies, Piscataway, NJ, USA). The cycle sequencing was performed as per manufacturer's instructions (ABI Prism Bigdye Terminator Kit version 2.0, Applied Bio Systems, Foster City, CA, USA).

RESULTS

From 862 Saudi subjects screened for G6PD deficiency by spectrophotometric assay, 47 individuals (35 men and 12 women, with a median age 26 years and men : women 3 : 1 ratio) were found suffering from G6PD deficiency. The corresponding frequency detected as 5.1% with 8.1 and 2.8% break in two genders.

Exons 6 and 7 of G6PD gene from 47 G6PD deficient cases were analyzed for sequence variations in comparison with normal individuals using CSGE. Twenty-five of G6PD deficient samples were found to have abnormal CSGE patterns, corresponding to G6PD Mediterranean variant (n = 24) and Sibari G6PD variant (n = 1). The results showed that most common G6PD mutation in this study was Mediterranean type (51.1%).

Conformation sensitive gel electrophoresis analysis of exon 6 PCR products was able to demonstrate aberrant CSGE patterns in 25 G6PD-deficient subjects (Figure 1). Direct DNA sequence analysis of the amplified PCR products revealed C→T substitution at codon 188 of G6PD gene (Figure 2). In addition, an A→G transition at nucleotide 634 of exon 6 (ATG-GTG; codon 212) was identified in one case, which indicates that this variant is known to be as Sibari type (Figure 3).

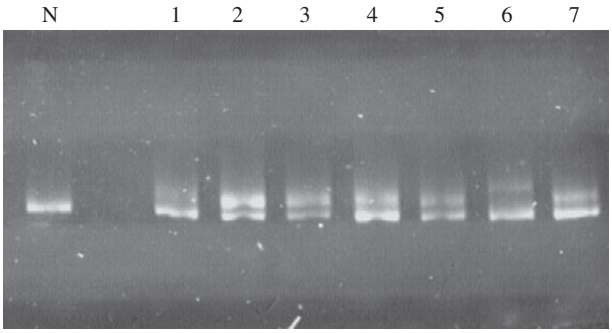

Figure 1. CSGE analysis of the PCR products for exon 6 of the G6PD gene. The CSGE gel shows exon 6 (364 bp) amplified from G6PD cases (lanes 1–7) displaying an abnormal CSGE profile compared with a normal (lane N).

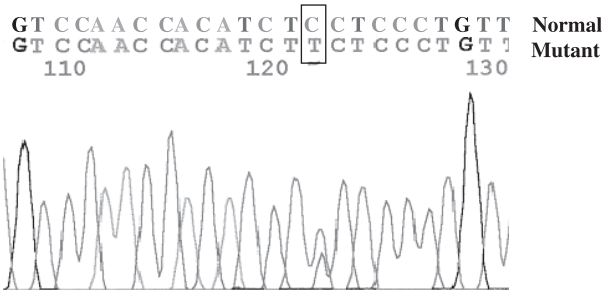

Figure 2. Sequence analysis of exon 6 of G6PD gene. The box indicates C–T nucleotide alteration leading to a change of amino acid Serine to Phenyl Alanine at codon 188 characteristic of Mediterranean mutation.

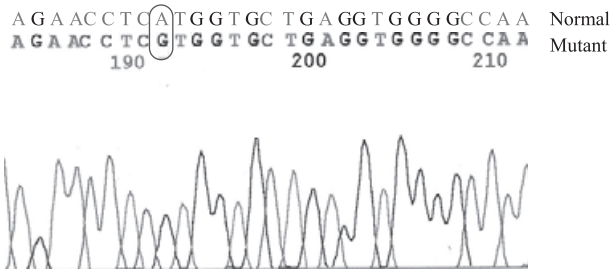

Figure 3. Sequence analysis of PCR product exon 6 of G6PD gene. The box indicates an A–G nucleotide alteration leading to a change in amino acid Methionine to Valine at codon 212 characteristic of Sibari mutation.

DISCUSSION

As standard procedure laid out for characterizing G6PD (Kirkman *et al.*, 1967), a considerable genetic heterogeneity at have been widely reported (Beutler & Yoshida, 1988; Butler and Vulliamy, 2002). These mutations have been found in the coding region of the G6PD gene and almost all of them are single base substitutions leading to an amino acid replacement (Tishkoff *et al.*, 2001). The two mutations G6PD Mediterranean and G6PD Sibiri with different polymorphic rates reinstate earlier studies. The G6PD Mediterranean, having C→T transition at nucleotide 563 of exon 6 with most prevalent allele has been reported from Mediterranean Middle East and India as well (Kurdi-Haidar *et al.*, 1990). However, the incidence of this mutation is much higher than this study (Bayoumi *et al.*, 1996; Al-Ali *et al.*, 2002; Alfadhli *et al.*, 2005). Thus, the frequency estimates of Mediterranean mutation ranges between 71.4% and 84% for UAE, Oman, Kuwait, and eastern Saudi Arabia (Bayoumi *et al.*, 1996; Daar *et al.*, 1996; Al-Ali *et al.*, 2002; Alfadhli *et al.*, 2005). The corresponding figure for Egyptian population is to the tune of 2–9% (Kamal *et al.*, 1967; McCaffery & Awny, 1970; Selim *et al.*, 1974; Rizk *et al.*, 2000). Similarly, a higher incidence rate; approximately 70% is reported in countries closer to the Mediterranean region, (Martinez di Montemuros *et al.*, 1997), while in Kurdish Jews, it is as high as 80–97% (Oppenheim *et al.*, 1993).

It is a well-established fact that marked linkage disequilibrium exists between silent polymorphic sites with coding sequence polymorphisms (Beutler, 1996). The Mediterranean mutation in Europe and Middle East is in unison to this report as it is associated with a silent C→T transition at nucleotide position 1311 of the G6PD gene while, for Italy and India, it is a rare 1311C genotype (Beutler, 1996; Beutler and Kuhl, 1990; Sukumar *et al.*, 2004).

In comparison, Sibari mutation is a rare mutation in Saudi Arabia but quite common in Italian population (Beutler, 1994). As the patient with an only Sibari mutation in this case was originally from Southern region of Saudi Arabia, further analysis could not be performed as the Details of patient's ethnic origin were not available. However, finding a Sibari mutation in the Saudi population does reflect a considerable genetic heterogeneity in Western region. This is expected given to special geographical position of Jeddah a meeting place of three continents having people from different ethnic groups. A detailed molecular evaluation of G6PD variants is further proposed for entire Saudi Arabia, and the work on these lines is in progress.

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