ORIGINAL ARTICLE

Investigation of Cosenza Mutation in Patients with Deficiency of Glucose-6-Phosphate Dehydrogenase (G6PD) in North West of Iran

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(Received: 12 October 2014 Accepted: 16 December 2014)

ABSTRACT: Glucose-6-phosphate dehydrogenase (G6PD) is a greatly polymorphic enzyme encoded by human X-linked gene. G6PD deficit is the most public enzymopathy in human with about 400 million people affected globally. It is the main controlling enzyme in the hexose monophosphate shunt catalase the oxidation of glucose-6-phosphate to 6-phosphogluconolacton and the creation of reducing equals in the form of NADPH to meet the cellular redox formal and its absence origin hemolytic anemia - favism and newborn jaundice. Mutation in this enzyme cause three major types of unusual phenotype, including Mediterranean, Chatham and Cosenza. In this study, by Rapid Genomic DNA Extraction (RGDE) method, from 90 blood samples of unrelated male and female patients with genetic deficiency of G6PD, DNA was removed and next digestion by Eco8I enzymes, in order to research for Cosenza mutation, they were analyzed by means of PCR-RFLP. Sequencing methods were used. Of 90 patients, one patient had a Cosenza mutation frequency of 1.01%. Eighty-nine patients (98.99%) were not affected by the Cosenza-type mutation. Accordingly, Cosenza mutation is not regarded as the most common mutation in Iranian North-west population.

INTRODUCTION

The enzyme G6PD (Glucose-6-phosphate Dehydrogenase) is one of the most significant enzymes in human body, which dissimilar cells in human body counting red blood cells have variable extents of the enzyme [1]. G6PD catalyzes the initially reaction of the pentose phosphate cycle (PPC) via which glucose6-
phosphate (G6P) is oxidized to 6-phosphogluconolactone with creation of NADPH [2-4]. Pentose phosphate cycle alters glucose into pentose phosphates and products reducing power in the form of NADPH. In RBCs, NADPH is vital for the defense against oxidative stress [5]. A main role of NADPH is to preserve reduced glutathione (GSH) at a proportion larger than 500:1 over the oxidized form GSSG [6, 7]. GSH shows an important role in antioxidant protection via reacting with H₂O₂ and organic peroxides as well as by preservingthiol groups of Hb, another proteins and enzymes in the reduced state. H₂O₂ can be too cleansed by catalase, which is also steadied by strongly bound NADPH [8, 9].

The main clinical appearances of G6PD lack are acute hemolytic anemia, neonatal jaundice, mental retardation, chronic renal failure, and chronic nonspherocytic hemolytic anemia. In children or adults with G6PD lack using of some elements such as Anti-malarial drugs, oxidizing materials or fava beans sources severe hemolytic attacks which can be life-threatening [10,11]. The gene coding G6PD is placed on the long arm of the X chromosome (Xq28) and involves of 12 introns and 13 exons, straddling nearly 18 kb. The coding sequencing of the gene is 1,545 bp long that coding for the 515 amino acids of the G6PD main sequence [12]. Outstanding to the position of genes, heredity of G6PD is an X-linked pattern. Hereafter, the imperfect gene in men is entirely out broken. In females due to requiring two X chromosomes, diverse forms of homozygote and heterozygote are observable [13].

G6PD enzyme deficiency is one of the greatest common human genetic illnesses so that more than 400 million people are affected worldwide [14]. Although Iranian population contains of unlike ethnic groups, but the total incidence of G6PD deficiency in Iranian population is assessed about 10%-14.9% [15]. Some studies were carried out on the molecular basis of G6PD lack in Iran and displayed that the Mediterranean mutation have the peak frequency in Gilan, Mazandaran, Golestan[16], Khorasan, Sistan & Baluchestan, Yazd and Kerman[15].In the greatest cases shift of a base at the DNA level, causes the shift of one amino acid with another amino acid. Standard type G6PD is identified type B. Conferring to preceding studies; the greatest widespread mutations in Iran are Mediterranean, Chatham and Cosenza. The goal of this study was to investigates the frequency rate of the Cosenza mutation in north-west of Iran.

**MATERIALS AND METHODS**

In this study, 90 peripheral blood samples (2-5 ml in 300 μ EDTA 0.5 M) from distinct patients with G6PD deficiency were collected from the hospitals of the north-west of Iran (including Ardebil, Tabriz and Urmia provinces). The blood samples collection method was performed with the ethics group approval and the patients’ informed consensus through telephone correspondences. Until the DNA extraction, the falcons were held in the temperature of -20°C. Qualitative amount of the enzyme activity was done using Fluorescent Spot Test and Saba laboratory kit. The basis of this method is the catalytic activity of G6PD enzyme in alters glucose 6-phosphate to 6-Phosphogluconate and real-time renewal of NADP to NADPH₂. The created NADPH₂ has fluorescent qualifications under UV (365nm). The fluorescence amount in the blood of healthy individuals is positive (strong) and in the blood of patients with G6PD deficiency is low or negative.

Extraction and purification of the samples’ DNA Genomic, from peripheral blood leukocytes, was achieved by Rapid Genomic DNA Extraction (RGDE) method. RGDE method was achieved as chart [17] as follows: Pouring 500μl or 0.5 gr of blood sample into a 1.5 ml microfuge tube and 1000µl of Cell Lysis buffer. Shaking microfuge tube mildly, and then centrifuging it for 2 minutes at 6000 rpm. Eliminating and discarding supernatant and repeating steps 1-3 two or three. Adding
300µl of Nuclei Lysis buffer to the microfuge tube and keeping the tube in room temperature for 2 minutes to prevent clot formation. Adding 100µl of saturated NaCl and 600µl of Chloroform to the microfuge tube, shaking it gently then centrifuging it for 2 minutes at 6000rpm. Transferring 300-400µl of supernatant to a new 1.5ml microfuge tube. Adding 600µl of cold Isopropanol to it; shaking it softly then rapidly. Then centrifuging the microfuge tube for one minute at 13000 rpm to precipitate, and then eliminating some of supernatant and allowing entirely dried in room temperature and care it for future uses.

Extension of exon 6 from the G6PD gene was done by primers Cosen-F [5'GCAGGCGATGGCAGCAGCAG 3'] and Cosen-R [5'GGGAAGGAGGGTGCGCCGG3'] 92 in thermal cycler (Senso Quest-Germany).

For each topic patient, 3µl of the stated primers, DFS-Taqmaster mix 12/5 µl was added to the PCR tube. Then 2µl of the DNA was added to the tube and until the tube’s final volume 25µl purified water (dH2O) was added to it. PCR situations were as follows: Primary denaturation of DNA at 94˚C for 5 min. Thirty five cycles each involving of three stages of denaturation at 94˚C for 1 min, annealing at 59˚C for 1 min, extension at 72˚C for 1 minute and final extension at 72˚C for 5 minutes.

PCR Products were electrophoresed on 1.5%-2% agarose gel at occurrence of DNA Ladder 50-700bp (Bioron-Germany) and negative control samples (Figure 1).

To study the G6PD genetic changes (exon 6) correctly, DNA sequencing was done in a casually selected sample of Cosenza Mutant. To do consequently the PCR product was sent to Fazapazhouh Company (USA) and the reply was analyzed by means of Chromas Lite version 2.33 software (Figure 3). This study was showed on 90 individuals with deficient G6PD enzyme activity. Individuals’ ages reached from one month to 21 years. G6PD Cosenza mutation (1376G-C) was observed in one case, which signifies the incidence rate of 1.01%. Overall, 89 patients (98.99%) were not affected by the Cosenza type mutation. Therefore, we conclude that this mutation is rare in Northwest of Iran and many cases associated with other mutations. Cosenza fault occurrence rate was 100% in men and 0% in women and it displays the high frequency of this disease in males and X-Linked recessive pattern of inheritance.

RESULTS

After electrophoresis on 1.5-2% agarose gel, in cases of the absence of Cosenza mutation and the enzymatic digestion, 548 bp band were visible. If there was a mutation, 548 bp band was cut and turned to 232 bp and 316 bp (Figure 2).

Figure 1. The results of amplification of nucleotide sequence containing G6PD deficiency without Cosenza mutation. Lanes 1-12: PCR product. M: 50bp plus DNA Ladder

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DISCUSSION

Using RFLP-PCR (DNA extracted and cut by special restriction enzymes), this study showed that 1.01% of individuals with Cosenza type enzyme absence have mutations in nucleotide 1376 (G-C) of G6PD gene. In these individuals, at position 459, arginine amino acid is replaced by proline.

G6PD is one of the most common genetic diseases that numerous people in the world suffer from it. This disease has produced many damages on child growth and it has taken into deliberation by the World Health Organization. Based on altered studies, Cosenza mutation occurrence is different round the world and Iran.

G6PDCosenzamutation observed for the first time in Cosenza state with rate 2.8% in Southern Italy, although the frequency of this mutation in other regions of Italy was reported 1.2% and 1.9%. In Italy, the rate of this mutation was reported 1.2% [18]. The highest frequency for Cosenza mutation was in Hormozgan with 12.33% and the lowest was in Fars with 0%. In 2007, based on a study conducted in Mazandaran the rate of Cosenza
mutation has been reported 6.75% [19]. In West of Iran, the rate of this mutation was 1.5 % (Among people 14-18 years) [20]. Noori-Daloii et al. Studies conducted in Hormozgan, Fars and Esfahan in 2006, showed that respectively 12.33%, 0% and 0% of patient shad Cosenza mutation [21, 22]. In Khuzestan Province in 2011 rate of this mutation was 2.6%. In Palestine mutation rate is 0%, although the results obtained from our study is completely consistent with the result but can be expressed that the results adapted together (because the same gene pool and geographical location) [23]. In the present study, prevalence rate of the mutation is 1.01% which, related to the described mutations, signifies the lowest prevalence rate. This study and the previous studies, showed in the provinces, disclose that Cosenza mutation type has the lowest prevalence rates among the three variants mentioned. Numerous studies have exposed those patients with G6PD deficiency are resilient against infectious malaria [24]. Since the prevalence rate of malaria in the northwest of the country is lower than the South and Southeast regions, so the prevalence of deficiency for this region is reasonable. While, due to the inheritance of X-linked recessive, it is predictable that this deficiency should be detected only in males but based on the Lyon’s hypothesis (random inactivation of sex chromosomes), though less widespread, it is likely to happen in females but in this study because statistical society selected was small, this mutation has not been observed in female.

CONCLUSIONS

Cosenza mutation has the lowest prevalence rate in northwest of Iran and most mutations are related to two other types.

ACKNOWLEDGMENTS

We would like to appreciate the respected authorities of the Hematology Laboratory of Koodakan-E-Tabriz, Bu Ali Ardabil and shahid Motahari hospitals to give samples and also we would like to appreciate authorities of the Islamic Azad University of Marand to agree for the use of apparatus and services of Genetics Laboratory. The authors declare that there is no conflict of interests.

REFERENCES