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Molecular Characterization of Glucose-6-phosphate Dehydrogenase Deficiency in Oman

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ABSTRACT

Objectives: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most encountered abnormality of red blood cell metabolism worldwide and has a high prevalence in Oman. The objective of the study was to characterize the mutation variants of G6PD deficiency in a cohort of the Omani population with partial and complete enzyme deficiency. ***Methods***: This prospective study included newborns and children less than one year of age with partial or complete G6PD enzyme deficiency identified on routine screening using a fluorescent spot test from 31 January 2017 to 12 September 2017 in Sultan Qaboos University Hospital. The identified samples were analyzed for the presence of C563T, G1003A, and other mutations using direct DNA sequencing of the polymerase chain reaction. ***Results:*** Out of 3679 newborn samples screened, 21.0% were found to have complete or partial G6PD enzyme deficiency. A total of 145 participants were included in the genetic analysis, of which 133 (91.7%) were completely deficient in G6PD enzyme activity and 12 (8.3%) had partial deficiency. The Mediterranean variant (C563T) was identified in 129 (89.0%). Other variants were found as follows: eight (5.5%) had variant A-, three (2.1%) had the Chatham variant (G1003A), one (0.7%) had the Cosenza variant, and one (0.7%) had exon 11 variant. No mutation was found in two subjects. ***Conclusions:*** The most common mutation in the Omani population is the Mediterranean mutation (C563T) followed by the variant A- mutation. However, not all participants were found to have a mutation.

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme of the pentose shunt pathway which catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucoanate. A byproduct of this reaction is NADP which is an essential component for different chemical reactions in the pathway, importantly the reaction involved in the production of reduced glutathione. G6PD enzyme is the only source of NADP in the pentose shunt pathway. Hence, the deficiency of the G6PD enzyme renders the red cell susceptible to oxidant stress.[1]

G6PD deficiency is an X-linked inherited disorder that results from a mutation in the G6PD gene. It is the most frequently encountered disorder of red blood cell metabolism, affecting > 400 million people worldwide. It is common in many ethnic groups including many African populations, Afro-Caribbeans, and African Americans, populations around the Mediterranean basin, Middle-Eastern populations, Indian subcontinent, and Southeast Asia, and Papua New Guinea.[2] In a previous population study in Oman, the prevalence of G6PD enzyme deficiency was found to be 25% in males and 10% in females.[3]

A wide variety of mutations in the G6PD gene have been identified, of which the most common forms are the Mediterranean variant (C563T), the Chatham variant (G1003A), and the A- variants (G202A in exon 4 and A376G in exon 5), which occur with increased frequency in certain populations. Recent studies from Iran and Iraq showed that the Mediterranean (563 C→T), Chatham (1003 G→A), and A- (202 G→A) mutations are responsible for most of the mutations in that region.[4–6] Another study conducted in a selected population in Oman

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with a small sample size revealed similar results.[7] We aimed to evaluate the prevalence of different G6PD gene mutations in a cohort of Omani patients with G6PD enzyme deficiency. We also evaluated the incidence of G6PD deficiency in Sultan Qaboos University Hospital (SQUH).

METHODS

This prospective study was carried out at SQUH from 31 January 2017 to 12 September 2017. Blood samples were obtained from the routine tests requested for newborns and children less than one year old for G6PD deficiency and found to be deficient or partially deficient after receiving the written informed consent from the parent or guardian of the newborn. Given that no additional blood samples were collected from the patients, no physical risk to the patient was associated with the study.

G6PD deficient patients were identified using Randox fluorescent spot test (Randox, Antrim, UK). This is a qualitative assay that requires incubation of patient blood with glucose-6-phosphate and NADP, a reaction that produces NADPH as a byproduct which fluoresces when examined under long-wave UV light. At our institution, this test is performed routinely for all newborns using 4 mL of fresh blood collected in a K2EDTA tube. Normal and partially deficient controls are tested in parallel with patient samples. Samples that result in deficiency or partial deficiency of G6PD activity were selected for mutation analysis and 2 mL of the remaining blood was used for the G6PD gene mutation variant analysis.

Genomic DNA was isolated from peripheral blood samples using the commercial blood mini extraction kit (Qiagen, Inc., Valencia, CA, USA). All samples were analyzed for the presence of the C563T (Mediterranean) in exons 6 and 7 using the direct DNA sequencing of the polymerase chain reaction.

If the C563T mutation was not identified, the second commonest mutation Chatham (G1003A) in exon 9 was screened. However, if the G1003A mutation was not identified, then other mutations with known association to a deficient phenotype were screened which include variant A- (G202A in exons 3 and 4, and A376G in exon 5), Cosenza (G1466C in exon 12) and exon 11 (T1399C). If no mutation was found, then the entire G6PD gene segment (including the promoter, all exons, and exon-intron junctions) was screened.

The polymerase chain reaction products were purified with EXO-SAP (USB, Cleveland, OH, USA) and cycle-sequenced with the ABI 3130 analyzer by utilizing the BigDye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA).

The data was analyzed by SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The mean and median were used for continuous variables and the percentage was used to describe the categorical variables.

RESULTS

A total of 3679 G6PD deficiency screening tests were requested from 31 January 2017 to 12 September 2017; 715 (19.4%) were found to be deficient for G6PD enzyme and 59 (1.6%) were partially deficient.

A total of 145 participants were screened for genetic mutation with a median age of one day (range: 1–365 days). There were 110 males and 35 females [Table 1]. Complete and partial enzyme deficiency were seen in 133 participants (91.7%) and 12 participants (8.3%), respectively. Among males, 79.7% had complete enzyme deficiency whereas this was 20.3% among females. The mean hemoglobin level was 15.1±2.0 g/dL.

One hundred and twenty-nine participants (89.0%) demonstrated the Mediterranean variant (C563T), of which 98 were males and 31 were female. Among patients with complete G6PD

Table 1: Baseline characteristics of participants.

Characteristics	All patients n (%)
Age, median (min and max), days	1 (1 and 365)
Gender	145 (100)
Male	110 (75.9)
Female	35 (24.1)
G6PD status, complete deficient	133 (91.7)
Male	106 (79.7)
Female	27 (20.3)
Partial deficient	12 (8.3)
Male	4 (33.3)
Female	8 (66.7)

deficiency, 94% had the Mediterranean variant while among those with partial deficiency, 33% had this variant. Other variants were found as follows: eight (5.5%) had variant A-, three (2.1%) had Chatham variant (G1003A), one (0.7%) had Cosenza variant and one (0.7%) had exon 11 variant. No mutation was found in two female participants with partial deficiency.

DISCUSSION

Among newborns screened for G6PD enzyme deficiency during the study period at SQUH, the incidence of G6PD deficiency was 21.0%. Among the analyzed samples, the Mediterranean variant was the most common followed by variant A-, Chatham, Cosenza, and exon 11. No mutation was found in two females with partial deficiency.

In one report, the frequency of G6PD deficiency was 26% in a male student population.[7] In a survey conducted by Al-Riyami et al,[3] in 2003, the frequency was 25% among males and 10% among females.In the regional countries, G6PD deficiency ranged in frequency from 22.3% in Bahrain[8] to 7.4% and 4.7% in the UAE[9] and Saudi Arabia,[10] respectively.

Most of the mutations found in the coding region of the G6PD gene are single-base substitutions, leading to an amino acid replacement.[11] The Mediterranean variant having C to T transition at nucleotide 563 of exon 6 had been reported in previous studies from the Middle East population.[12] Many countries in the region including the UAE,[9] Kuwait,[13] Saudi Arabia,[10,14] Iran,[15] and Iraq[5] reported a high prevalence of the Mediterranean variant range from 74% to 91%. In our study, a similar prevalence of the Mediterranean variant of 89.0% was identified.

The second commonest mutation in our study is variant A-, in which G is substituted by A at nucleotide 202 in exons 3 and 4, as well as G substitutes A at nucleotide 376 in exon 5. It had been reported previously in the Middle East but with a low rate and does not appear to be a major cause of G6PD deficiency in this region.[10,13] We report a higher rate of variant A-.

Other mutations described for G6PD enzyme deficiency include Chatham, Cosenza, and exon 11 variants. From the results of our study, these appear to play no major role in causing G6PD deficiency in Oman. However, Daar et al,[7] reported a higher prevalence of the Chatham variant mainly due to selection bias and the smaller sample size. Cosenza variant has been reported previously in Iran.[4] Interestingly, no mutations were found in two samples after screening the promoter, all exons, and exon-intron junctions. They were all female participants who had partial G6PD deficiency. Further tests are needed to be done including repeat G6PD assay to confirm the G6PD status and repeat the genetic tests.

This generalizability of the study is limited due to the single-center design and therefore, may not represent the Omani population. It can be the basis for future research to correlate the G6PD genotype and clinical severity. In conclusion, the Mediterranean variant is the predominant mutation variant in Oman. However, variant A- is a higher rate in Oman comparing to nearby countries.

CONCLUSION

Mediterranean (C563T) followed by variant A- mutations are the commonest mutations causing G6PD deficiency in the Omani population. Not all patients were found to have a mutation and therefore, we recommend a more comprehensive genetic testing in the future studies addressing this question.

Disclosure

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