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Prevalence of glucose-6-phosphate dehydrogenase deficiency in newborns in northeast Mexico


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Conflict of Interest: The authors declare that they have no conflict of interest.

Abstract:
Background: Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is the most common enzymatic disease worldwide and the prevalence is not well established because of the lack of screening.
Objective: This study aimed to estimate the prevalence of G6PDd in a Hispanic population from Northeast Mexico.
Study Design: In this retrospective study, a database was used to analyze the G6PDd in neonates included in the expanded newborn metabolic screening of inherited metabolic disorders during a period of four years through the GSP Neonatal G6 kit (PerkinElmer).
Results: Among 96,152 (48,462 male) neonates screened for G6PD enzyme activity, a total of 566 (0.58%) cases were deficient for G6PD. Of those 566 patients, 469 (82.8%) attended the second test and the other 97 (17.2%) patients were lost. Of those 469 who did attend, 384 (81.9%) neonates were deficient in the second test and 85 (18.1%) were normal. With the data collected, 384 neonates were confirmed with G6PDd, 348 (88.6%) were male and 36 (11.4%) patients were female. The calculated prevalence for this population was 0.72 cases per 100 male newborns.
Conclusion: The prevalence of G6PDd in the Northeastern Mexican population is high. Since migration is increasing in the United States, pediatricians should be aware of the need to search for G6PDd in newborns and the wide clinical manifestations they can present.

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Prevalence of glucose-6-phosphate dehydrogenase deficiency in newborns in northeast Mexico

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**Conclusion:** The prevalence of G6PDd in the Northeastern Mexican population is high. Since migration is increasing in the United States, pediatricians should be aware of the need to search for G6PDd in newborns and the wide clinical manifestations they can present.

**Keywords:** Glucose-6-phosphate dehydrogenase, newborn, prevalence, neonatal screening

**Key Points**
The calculated prevalence of Glucose-6-phosphate dehydrogenase deficiency in northeast Mexico is 3.99 cases per 1000 newborns.

Glucose-6-phosphate dehydrogenase deficiency screenings should be included in all newborn metabolic screenings.

Glucose-6-phosphate dehydrogenase deficiency is a common erythroenzymopathy that must be addressed as a public health concern. To anticipate clinical complications, target population monitoring is required.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PDd) is the most common erythrocyte enzyme defect and X-linked disorder that affects more than 400 million people worldwide.\textsuperscript{1-2} G6PD is an enzymatic protein located in the cytoplasm of all human cells; however, the amount of the enzyme varies in different tissues.\textsuperscript{3} G6PD plays an important role in the pentose phosphate pathway. It produces the oxidation of glucose 6-phosphate into 6-phosphogluconolactone with the consequent reduction of NADP to NADPH which helps maintain normal glutathione levels to protect cells against oxidative stress.\textsuperscript{4}

Deficiency of G6PD causes a reduction in number of active enzyme molecules and decreases erythrocytes’ ability to respond to hydrogen peroxide oxidative stressors, therefore the erythrocyte membrane is more fragile and prone to rupture. In normal conditions the erythrocytes can perform normally, however, when exposed to different triggers such as infections, medical herbs, products, and foods they tend to suffer from oxidative stress and to induce hemolysis.\textsuperscript{5}

This is also explained because the enzymatic activity of G6PD is reduced as time passes, thus the enzymatic activity in mature erythrocytes is five times lower compared to reticulocytes.\textsuperscript{6}
The genetic etiology was first described in 1958, and because it is an X-linked disorder, patients may show different genotypes depending on their gender.\textsuperscript{7} 217 different mutations have been described up to 2016 for this particular enzyme, which makes it a heterogeneous disease.\textsuperscript{8} G6PDd has a broad clinical spectrum going from asymptomatic patients to different hemolytic syndromes such as acute hemolytic anemia, favism, congenital nonspherocytic hemolytic anemia, and neonatal hyperbilirubinemia, which in extreme cases can be manifested as kernicterus.\textsuperscript{4} Acute hemolytic conditions are triggered by external factors capable of producing oxidative stress such as certain drugs, chemicals, foods, infectious diseases, and metabolic abnormalities.\textsuperscript{4,9} In Mexico, the most frequent variants are the G6PDd A- and the G6PDd Santamaría, which represent approximately 82% of the patients affected.\textsuperscript{10} In a systematic review, Monteiro et al.\textsuperscript{11} reported a G6PDd prevalence in Mexico of 0-12.7% and a 0-0.6% prevalence not discriminated by gender. The aim of this study is to report the prevalence of G6PDd in the state of Nuevo Leon by analyzing the metabolic screening results of all neonates born in public hospitals between 2012 and 2015 and comparing it with studies previously reported in other regions.

\textbf{Methods}

\textit{Study design}

We conducted a retrospective study to analyze the results of the determination of G6PDd included in the applied testing to neonates included in the expanded newborn metabolic screening of the Secretary of Health of the State of Nuevo Leon from January 2012 to September 2015. This study was approved by the research ethics committee of the UANL School of Medicine with a number study of PE18-001.

\textit{Materials and methods}
We included all neonates born in the Secretary of Health Hospitals of Nuevo Leon, to whom the screening was performed, regardless of the gestational age, birth weight, or any other delivery complications. In our study 96,152 neonates were screened. The blood samples were obtained through the heel stick technique and collected in Guthrie cards which were kept in a cold chain and delivered by trained authorized staff for processing in the Biochemical Genetics Laboratory at Hospital Universitario “Dr. José Eleuterio González” located in Monterrey, Mexico. Samples are taken before discharge and are usually processed within 24 hours from arrival to the lab. After arrival to the lab, samples were processed and enzyme activity was quantified through immunofluorescence using the GSP Neonatal G6PD kit, based on the same enzymatic reaction as the manual, PerkinElmer Neonatal G6PD (ND-1000) assay. This reaction is temperature controlled and controls are used in every processed plate. This technique’s accuracy is measured with a coefficient of variation of 8.8%. To determine G6PD activity levels, the fluorescence of NADPH is measured using an excitation wavelength of 340n and an emission wavelength of 460 nm. The cut-off values of G6PD activity in male subjects below 1.6 U/g Hb and a value of G6PD activity below 1.9 U/g Hb in the female population were considered out of normality. After obtaining an initial deficient result on the screening test, a second test is performed in the same sample. After two deficient results in the same sample a 2-tier test will be taken and processed by the previously described method. Parents are informed about G6PDd alarm symptoms prior to discharge, as well as the importance of being available for a second sample if the first is deficient, in which case they will be contacted as soon as the lab has the result to confirm the diagnosis. After two abnormal samples, confirmed patients entered a follow-up program involving the Pediatric Hematology and the Clinical Genetics Department. During the appointments, patients were assessed with an initial blood count. Parents were informed about the disease
and received genetic counseling. Additionally, they were given a brochure containing the previous information and a list containing the foods and drugs that must be avoided.

**Statistical analysis**

SPSS software (version 24) was used to analyze data. Data are represented as numbers and percentages. A Receiving Operating Characteristic Curve (ROC curve) was used to calculate the sensitivity of the test, to help categorize whether or not the newborns had G6PDd. Using the false positive and the true positive rate of fluorescence NADPH in the “x” and “y” axis, respectively. The cut-off values of G6PD activity below 1.6 U/g Hb for males and 1.9 U/g Hb for females were considered out of normality. Because the test was not the main focus of this study, the area under the curve (AUC) and the Youden index were not calculated.

**Results**

A total of 96,152 neonates were screened for G6PD enzyme activity using the GSP Neonatal G6PD kit. 48,461 (50.4%) were male and 47,691 (49.6%) were female. From these 96,152, there were 566 (0.58%) cases that were deficient for G6PD. These 566 subjects were requested to repeat the test to confirm the diagnosis. Out of the 566 newborns that were deficient, 469 (82.8%) attended a second test. From these 469, 384 (81.9%) were deficient in the second test and 85 (18.1%) were normal. 97 (17.2%) from the original 566 that had deficiency did not attend a second test. (Fig. 1) With the data collected, 384 patients were confirmed with G6PD deficiency, from which 348 were male (88.6%) and 36 patients were female (11.4%). The calculated prevalence for the population not discriminated by gender was 0.39% and the prevalence in the male population was 0.72%. The ROC curve reported a cut off value below 1.6 U/g Hb in which a sensitivity of 81.87% was calculated.
Discussion

In 2018 the estimated coverage of newborn screening in Mexico was >84%. It occupied the sixth place preceded in order by Cuba, Costa Rica, Chile, Uruguay, and Argentina. Mexico lacks a unified national newborn screening program due to its complex health care system, that consists of multiple health service providers. Despite this, Mexico and Panama are the only two countries in Latin America that offer detection of G6PDd in their newborn screening program.\textsuperscript{12}

In Mexico the National Institute of Perinatology conducted a 5 year, single center study with a sample of 21,619 newborns that reported a prevalence of 1.89 cases per 1,000 newborns.\textsuperscript{14} Another study conducted by several hospitals in 18 states of Mexico of the SEMAR (Mexican Navy) between 2012 and 2014, used a sample of 5,205 newborns to report a prevalence of 0.96 cases per 1,000 newborns.\textsuperscript{15} Cantu-Reyna et al\textsuperscript{9} conducted a study in which they analyzed a total of 50,577 extended metabolic screenings (25,721 males) in the state of Nuevo Leon, Mexico between October 2005 and November 2015 and found a prevalence of 3.97 cases per 1,000 newborn males in the state of Nuevo Leon. Our results showed a closer frequency to this last one because it was done with a similar regional population. The disparities between these two studies and the previous ones indicate that the prevalence of this disease varies greatly among different populations, even within the same country. According to the systematic review by Monteiro et al\textsuperscript{11} the overall prevalence range among studies from Latin America not discriminated by gender is from 0-13.3%.

Unfortunately, facilities and services differ by area, and screening is not conducted in all states. This could lead to underestimate the real prevalence of G6PDd; nevertheless, data from our analysis could be used to calculate the prevalence in regions where G6PDd
screening is not available.\textsuperscript{9} The prevalence of G6PDd could be growing due to the increase in the rates of migration that occurs in the Americas.\textsuperscript{6,16}

In our hospital, the approximate cost of G6PDd screening test is equivalent to $2.50 USD, this can be considered to analyze the cost/benefit impact of screening every newborn, since some cases of G6PDd can lead to severe hyperbilirubinemia, acute bilirubin encephalopathy, kernicterus, and death. With the early identification of G6PDd these complications become easier to manage. After the analysis, we calculated the sensitivity of our study. A ROC curve was used reporting a cut off value below 1.6 U/g Hb in which a sensitivity of 81.87\% was calculated, while on other studies it is also established a sensitivity of 82.2\% with the same cut-off value.\textsuperscript{17}

The American Academy of Pediatrics clinical guidelines recommends only screening jaundiced infants who are receiving phototherapy and whose family history or ethnic/geographic origin suggest the likelihood of G6PDd or for an infant in whom the response to phototherapy is poor.\textsuperscript{18} However, the World Health Organization recommends the G6PD screening on newborns when male prevalence of deficiency is 3-5\%, and in a more recent guide, in countries with high prevalence of Malaria and other congenital blood diseases.\textsuperscript{19,20} Despite these recommendations, there are still cases of neurotoxicity and kernicterus reported. According to the National Quality Forum, which is part of the Agency of Healthcare Research and Quality, and the American Academy of Pediatrics, hyperbilirubinemia that leads to kernicterus must be viewed as a “never event” and is “largely preventable”.\textsuperscript{21} Considering the cost-benefit analysis, G6PDd testing should be made widely available or even consider making it mandatory for all neonates before they are discharged to facilitate management and prevent most complications.
G6PDd is only routinely done through the newborn screening programs in Pennsylvania and DC. Because the Hispanic population in the US is growing due to immigration, pediatricians should be aware of G6PDd symptoms and risk factors.\textsuperscript{22,23}

**Conclusion**

G6PDd is the most prevalent enzymatic disease worldwide. Unfortunately, the prevalence of this condition and the extent of its clinical consequences have not been properly measured in the Latin American population because the screening is not routinely performed in many regions. Further studies should be designed and carried out to assess the cost/benefit of screening infants for G6PDd.

**Conflict of Interest statement**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Acknowledgments**

None.

**References**


FIGURE 1 Flow chart describing the patients metabolic screening.
96,152 newborns screened

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97 (17.2%) patients were lost

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