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G6PD DEFICIENCY;
GLUCOSE 6 PHOSPHATE DEHYDROGENASE DEFICIENCY; THE CASE FOR MASS SCREENING IN PAKISTAN

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ABSTRACT… Context: Hyperbilirubinemia due to glucose 6 phosphate dehydrogenase (G6PD) deficiency can cause permanent neurological damage and death in neonates. Screening for the enzyme enables timely diagnosis and treatment in cases of G6PD related kernicterus. Knowledge of patient G6PD status is also important in treatment of malaria, a disease endemic in Pakistan. World Health Organization recommends mandatoryuse of primaquine for radical cure and eradication of malaria. Since, Primaquine, causes hemolysis in G6PD deficient cases, widespread adoption of the drug is viewed with caution. **Aims:** This study assessed frequency of G6PD deficiency in Pakistani noenates and examines the need for its screening based on local disease prevalence and malaria endemicity. **Settings and Design:** A cross sectional study was carried at Hematology Department, Army Medical College (NUST), in collaboration with Military Hospital, Rawalpindi, Pakistan.(January - August, 2011). **Methods and Material:** The frequency of G6PD deficiency in newborn population was determined by quantitative (spectrophotometric) method. Cord blood (2.5 ml blood in K3EDTA bottle) samples were obtained from 240 newborns (male: female 1.2:1) after informed consent from parents. **Statistical analysis used**: Data obtained was analyzed using SPSS Windows version 17. **Results**: Frequency of G6PD deficient cases was 4.2%. Among the ten G6PD deficient patients, six had severe enzyme deficiency (<10% enzyme activity). **Conclusions:** The local prevalence of G6PD deficiency and its potential complications qualify it as a disease that must be screened for. Also, prior knowledge of patient G6PD status enables the physician to revert to modified treatment regimen for malaria only in enzyme deficient cases and not otherwise.

Key words: G6PD, kernicterus, malaria, primaquine, hemolysis,

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INTRODUCTION

Glucose 6 phosphate dehydrogenase is the world's most common inherited enzymopathy with global disease burden scaling to approximately 400 million people.[1] The disease can be tested for, at the time of birth, by cord blood analysis.[2]

Newborn screening for the enzyme would help reduce kernicterus associated mortality. Also, since Pakistan lies in malaria endemic belt, knowledge of G6PD status enables the physician to use drugs (particularly primaqune) judiciously.

This study was conducted to test the G6PD status of newborn population and explore the need and relevance of a screening programme in our country. It was also examined whether G6PD deficiency fulfils the criteria of newborn screening.

SUBJECTS AND METHODS

Across-sectional study was conducted in Hematology Department, Army Medical College, National University of Sciences and Technology and Military Hospital Rawalpindi from January to August 2011. Non probability convenient sampling technique was used and 240 cases were evaluated (after informed consent from parents). The study was approved by institutional ethical review committee, and subjects were enrolled after taking informed consents from parents. The families were provided information related to G6PD deficiency, and they were advised to undergo extended family screening in the event of baby being G6PD deficient.

Cord blood was obtained from all newborns. All subjects having reticulocyte count greater than

6 percent were not considered for the study. Old samples (samples that were not been evaluated within 6 hours of sampling) were discarded and new ones were obtained.

For each evaluation, 2.5 ml of cord blood was taken in K3 EDTA (Ethylene diamine tetra acetic acid) bottle (manufactured in Italy) and gently mixed. Samples were stored at 4^0 C prior to analysis and all samples were analysed within 6 hours of collection. Prior to testing for G6PD, complete blood count (CBC), evaluation using Sysmex Hematology Analyzer KX-21 was done for each sample. The Hb concentration (g/dl) was used in subsequent calculations Evaluation of peripheral film (using Leishman stain) and reticulocyte count (using Brilliant Cresyl Blue) were also performed. This was done to rule out abnormally raised reticulocyte count (greater than 6 percent).

For G6PD quantitative analysis, the kit manufactured by AMP Diagnostics, Austria (BD6400-E V3.0 CE) was used. The reduction of NADP to NADPH in the reaction mixture was recorded at 340nm by a spectrophotometer. Microlab-200 (Merck, Netherlands) was utilized to record absorbance of reaction mixture. Absorbance values recorded at 5 minutes interval was denoted as A_1 and A_2respectively.Testwas performed as per manufacturer's instructions and G6PD activity was computed in U/g Hb.

For latest data on regional and local malaria statistics and World Health Organization treatment guidelines, the authors searched published English-language literature in PubMed and MEDLINE databases.

All data was analysed through the statistical packages for social sciences (SPSS), Windows version 17. Frequency of G6PD deficient cases, mean G6PD activity in males and females and standard deviations were also computed The 2 sample t-test was used to calculate the p value. At 95% confidence level, p-value less than 0.05 was taken as significant.

RESULTS

A total 240 newborns, conforming strictly to inclusion and exclusion criteria, were included in this study. Among these, 130 (54.2%) were male and 110 (45.8%), female. The meanG6PD activity for 130 male newborns was 16.42U/g Hb $\pm$ 4.04 and that for the 110 female newborns was 16.31U/g Hb $\pm$ 3.68.The p value was calculated, using 2 tailed student t test and it was noted that there was no significant difference (p value = 0.8) in this value between sexes. The collective mean value for 240 newborns was 16.37 U/g Hb $\pm$ 2.76.

According to the criteria set by World Health Organization, total enzyme deficiency is defined as 10 % and partial deficiency is defined as 10–60 % of normal enzyme activity. Based on this, the lower and upper limits for partial deficiency were 1.64 U/g Hb and 9.82 U/g Hb respectively (**Table-I).** Therefore, all subjects having G6PD activity below 1.64U/g Hb were classified as having severe enzymopathy.

Mean normal G6PD activity (U/g Hb)	Upper limit of total deficiency (10% of mean normal U/g Hb)	Upper limit of mild/partial deficiency (60 % of mean normal U/g Hb)
16.37	1.64	9.82

Table-I. Mean normal G6PD activity and values of upper and lower limits for partial G6PD deficiency as per WHO criteria (expressed in U/g Hb)

In the male population, G6PD deficiency was diagnosed in (6/130) 4.6 % of cases. Furthermore, it was noted that all these cases had severe enzyme deficiency G6PD activity in range of 0 – 1.29 U/g Hb.

In the female population, (4/110) 3.6 % cases were diagnosed as deficient by enzyme assay (Table-II). All these cases had partial enzyme deficiency with a range of 4.33 – 8.48 U/g Hb.

Gender	No of cases	No of cases with partial deficiency	No of cases with severe deficiency	G6PD deficient individuals (%)
Females	110	4	0	3.6
Males	130	0	6	4.6

Table-II. Number of cases with partial and severe G6PD deficiency in newborn male population, with DRT and Quantitative G6PD estimation

The net frequency of the enzymopathy in our population is 4.2%. All males diagnosed as deficient had severe enzyme deficiency and all female diagnosed as deficient had partial enzyme deficiency. Normal G6PD activity had a range of 10.85-21.85 U/g Hb in our population.

DISCUSSIONS

We analyzed 240 newborns for estimation of G6PD levels. The frequency of enzyme deficient cases was 4.2%. According to the recommendations of the WHO, all newborns should be screened in populations where the prevalence of G6PD deficiency is 3-5 percent in males.[3]

In Pakistan, most jaundiced neonates are discharged from hospitals as such. If the jaundice is not physiological, it results in clinical deterioration of the newborn. If a screening protocol for G6PD deficiency is in place, the disease can be managed (phototherapy/ exchange transfusion) and the neonate be detained till recovery**.** Moreover, once a case of G6PD is diagnosed, siblings of the newborn can also be tested. Family screening will bring to light any other individuals affected.

The question is, should G6PD screening be carried out at a larger scale in Pakistan?
Nussbaum and colleagues have discussed the criteria for initiating a newborn screening programme in their book, 'Genetics and Medicine.[4] They are as follows:

1. Treatment has to be available.
2. Early institution of treatment before symptoms manifest has been shown to reduce severity of illness.
3. Routine observation and physical examination will not reveal the disorder and so a test is required.
4. A rapid and economical laboratory test is available that is highly sensitive and specific.
5. The condition is frequent and serious enough to justify the expense of screening.

The G6PD situation in Pakistan, by and, large meets the above criteria. Though a cure does not exist for this disorder, but the severity and implications of kernicterus are valid reasons to initiate preventive measures. A retrospective study conducted at Agha Khan Hospital, Karachi in 2007 revealed that 256 out of 2811 (9.1 percent) babies hospitalized for neonatal jaundice were positive for G6PD deficiency[5]. Independent, single centre studies conducted in Peshawar and Lahore have shown the frequency of G6PD deficiency in jaundiced neonates to be 4- 14 percent.[6,7]

Regarding the fourth point in the criteria, various dye reduction tests available are inexpensive options to screen the population.

Based on the results of our study and other local statistics discussed previously, a comprehensive neonatal screening programme for G6PD deficiency should be implemented in Pakistan, especially in Punjab and Khyber Pakhtunkhwa where an incidence rate as high as 8.3% has been reported.[8]
Such measures were taken in Sardinia in 1971 (prevalence 7.5 percent) and Greece in 1977 (prevalence 4.5 percent), when screening for G6PD deficiency in newborns was made mandatory.[9] Mass newborn screening programme in Singapore has resulted in decreased incidence of kernicterus. Over a span of two decades there was only one reported case of kernicterus in G6PD deficient newborns.[10]

The reason why we chose G6PD quantitative estimation as opposed to conventional dye reduction tests is twofold. The shortcoming of most dye reduction tests is their inability to diagnose patients with partial enzyme deficiency. Due to this, patients who are heterozygotes for the enzymopathy are often misclassified as

normal. Quantitative enzyme estimation enables us to classify the patients as having complete or partial deficiency as the end point is not a colour change but an absolute value (expressed in U/g Hb) Secondly, it gives as an exact measure of the enzyme activity. Our results show that most of our patients diagnosed with G6PD deficiency had severe form of the enzymopathy (less than 10% of normal enzyme activity) i.e., < 1.64U/g Hb.

A recent study conducted to determine the genotypic variants of g6pd deficiency in our population revealed 563C-T (also known as G6PD Mediterranean) as the most common variant. Very low levels of G6PD enzyme activity are seen with the 563C-T variant[11]. The results of our study are in agreement with this as we found very low enzyme activity in majority of our G6PD deficient patients. This has important implications for such patients being treated for malaria. The hemolytic potential of 8 aminoquinolones in G6PD deficiency is well documented. Such cases first came to light in the early 1900's but it was no sooner than 1956 that the mechanism of hemolysis was traced down to an enzyme deficiency[12]. As the degree of hemolysis after primaquine administration depends on the type of G6PD variant, it is most pronounced with G6PD Mediterranean.[13] This is the polymorphism associated with severe enzymopathy (<10% of normal enzyme activity) and happens to be the most common form of G6PD deficiency in Pakistan.

Malaria continues to be a serious public health problem in our country. In 2010, 22% of the one million confirmed cases of malaria in the Eastern Mediterranean region came from Pakistan. Microscopy and PCR studies confirmed plasmodium vivax infection in 76% of cases[14]. Statistics show that approximately 500,000 malaria infectionsand 50,000 malaria-attributable deaths occur each year in Pakistan.[15]

Pakistan Directorate of Malaria Control collaborated with WHO and a technical core group to formulate national treatment guidelines for malaria in 2005[16]. In addition to this, the 2010 WHO guidelines state that the treatment for uncomplicated *Plasmodiumvivax* malaria must incorporate primaquine.[17]

Numerous studies have shown,however, that primaquine can precipitate a life threatening hemolysis in patients with very low G6PD activity, i.e. patients with G6PD Mediterranean. According to recent guidelines of the World Health Organization, widespread use of primaquine in malaria endemic zones is essential for radical cure and effective eradication of malaria. Primaquine is the only licensed drug that has gametocydal properties and also eradicates hypnozoite stages of plasmodium.[18]

The disease burden of malaria on our resources is enormous. As primaquine is not prescribed as often as it should be, the number of Plasomodium vivax relapse cases is on the rise. Apart from unavailability, one of the reasons why physicians exercise caution in using primaquine is that they are unaware of patient G6PD status. In fact some ethnic communities in Pakistan have a G6PD prevalence rate as high as 8%.[8] The flip side of this problem is that due to this uncertainty, many malaria patients with normal G6PD activity are not prescribed primaquine.

This is where screening for G6PD deficiency becomes extremely relevant. If the G6PD profile of an individual is documented, physicians can treat for malaria as per WHO guidelines. Furthermore, recent studies have showed that a modified primaquine dosing regimen (0.25 mg base/kg) may be safe in G6PD deficient cases.[19]

It must be emphasized here that the ideal screening test for G6PD deficiency should be one that is cost effective, easy to perform and suitable for field use. Although the quantitative estimation of G6PD is very reliable, it does not fit the bill. In such a scenario, the Dye Reduction Test is a suitable tool for screening purposes. It is easy to perform, requires no special equipment and is cost effective.[5]

A study conducted in Uganda (malaria endemic zone) evaluated a recently developed G6PD

screening test (WST8/1-methoxy PMS method) designed for population screening and field use.[20] When compared to a gold standard enzymatic assay, it showed a sensitivity of 72% and specificity of 98%.

The focus in Pakistan should be to develop such tests that are suitable for mass screening. Also the inclusion of this test in malaria elimination programmes,contemplating the use of primaquine, will ensure effective case management on an individual level and guide plasmodium vivax treatment policy at a population level.With problems like artemesinin resistance posing new challenges in the fight against malaria, primaquine will help curb transmission and relapse rates.

G6PD testing is essential in this equation.

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"A real friend is one who walks in when the rest of the world walks out."

Walter Winchell

AUTHORSHIP AND CONTRIBUTION DECLARATION

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