Screening of Glucose-6-Phosphate Dehydrogenase Deficiency in Neonatal Hyperbilirubinaemia at 300-Bedded Pyin Oo Lwin General Hospital

Khin Moe Aung[1], Myint Myint Khaing[1], Tin Moe Khaing[1], Su Su Myaing[1], Htay Htay Kywe[1], Moe Kyaw Myint[1], May Thet Naing[2] & Win Aung[1]*

[1]Department of Medical Research, Pyin Oo Lwin Branch
[2]Department of Medical Services

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common human enzyme deficiencies in the world. It is particularly common in populations living in malaria-endemic areas, affecting more than 400 million people worldwide. This hospital- and laboratory-based, cross-sectional descriptive study was conducted with the aim of determining the prevalence of G6PD deficiency among 200 newborns at 300-bedded Pyin Oo Lwin General Hospital during January to March 2017**.** The participants were 103 girls (58.5%) and 97 boys (41.5%). Both qualitative and quantitative measurements by using Brewer's method and G-SIX kit method were applied for diagnosis of G6PD deficiency. Total serum bilirubin level was measured by Bilirubinometer. Of the 200 newborns, 21(10.5%) were G6PD deficient. The overall prevalence of G6PD deficiency was 10.5% (21/200) and male was predominant than female (17.5% vs 3.9%). Out of 10.5% (21/100) G6PD deficient newborns, 5(23.8%) and 16(76.2%) were mild and moderate G6PD deficiency, respectively. Regarding hyperbilirubinaemia, 9(42.9%), 3(14.3%), 2(19.0%) and 5(23.8%) were severe, moderate and mild hyperbilirubinaemia and normal bilirubin, respectively. This study showed that a significant correlation between the severity of hyperbilirubinaemia and G6PD activity ($p < 0.05$). Taking into consideration of the above results, the high prevalence can be useful for providing appropriate prevention and early treatment of complications in routine neonatal screening in this area.

Keywords: G6PD, Newborns, Brewer test, G-SIX kit

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common clinically significant red cell enzymopathy in humans, affecting around 400 million people world wide. The main clinical expression is a haemolytic anaemia, which can be acute or chronic. During the neonatal period, the disease may manifestas neonatal jaundice with pathological hyperbilirubinaemia which may develop sufficiently severe to cause kernicterus and even death or cerebral palsy.[1] According to World Health Organization, 7.5% of world population were carriers of G6PD deficiency and 2.9% were G6PD deficient.[2] It is most commonly prevalent in Africa, Southeast Asian and Middle Eastern populations.[3] With about 130 million births annually, about 4.5 million of G6PD-deficient children are particularly vulnerable to neonatal jaundice and acute hemolytic crisis. Owing to global migrations, the complications of G6PD deficiency may now occur in at least certain population groups in

*To whom correspondence should be addressed.
Tel: + 95-95156625
E-mail: drkhinmoeaungdmr@gmail.com
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most countries and regions of the world.[4] G6PD deficiency is a recessive X link trait, placing males at highest risk for symptomatic disease. G6PD deficiency manifests in several distinct clinical patterns. Several hundred G6PD genetic variants are known, but most are harmless. Only two variants, designated G6PD A - and G6PD Mediterranean cause most clinically significant haemolytic anaemia. G6PD is present in about 10% American blacks, G6PD Mediterranean, as the name implies, is present in the Middle East.[5] G6PD is a "house-keeping" enzyme, vital for the life of every cell. Complete absence of the enzyme is unknown in human species. G6PD catalyzes the initial step in the pentose phosphate pathway. It is important to note that though X-linked disorders are usually thought to effect males only, in this case, because of the high frequency of the gene and the high incidence of consanguineous marriages, homozygous females contribute about 10% of those genetically G6PD deficiency.[4]

Various studies had been done on G6PD deficiency and hyperbilirubinaemia globally. One study in Taiwan demonstrated that the lower G6PD enzyme activity was associated with the neonatal hyperbilirubinaemia in G6PD-deficient male neonates and finally suggested that the G6PD-deficient neonates are at increased risk for hyperbilirubinaemia even in the nursery free from agents that can potentially cause hemolysis to G6PD-deficient red cells.[6] Another study on neonatal jaundice and severity of G6PD deficiency in Sardinian babies in Italy showed that the residual G6PD enzyme activity was not significantly lower in G6PD-deficient babies with neonatal jaundice compared to G6PD-deficient babies without neonatal jaundice.[7] In Myanmar, the study on factors related to the severity of hyperbilirubinemia in newborn babies admitted to Special Care Baby Unit (SCBU) of Mandalay Children's Hospital had been concluded that G6PD deficiency was one of the most frequent risk factors for hyperbilirubinemia.[8] Severe neonatal jaundice, the most dangerous consequence of G6PD deficiency, was proved to be the most common clinical manifestation and globally important.[9] Early detection of G6PD deficiency with appropriate management can prevent mental handicap and related developmental disabilities.[10]

The early characterrization of G6PD activity provides an etiological diagnosis for neonatal jaundice, as well as the opportunity to give the newborn's family information concerning haemolytic crisis prevention. There is little study of G6PD deficiency in neonatal jaundice at Pyin Oo Lwin General Hospital. From this study, there may be possible ways of reducing the morbidity associated with neonatal jaundice due to G6PD deficiency. Early detection of G6PD deficiency can prevent the acute attack of hemolysis by avoiding the exposure to drugs and chemicals known to trigger hemolysis.

By detecting the severity of G6PD deficiency, the high risk neonates can be determined so that health education can be given to mothers of G6PD-deficient neonates for lifelong awareness of their babies for avoidance of precipitating factors which are very important and essential for life. Therefore, from this study, there may be possible ways of reducing the morbidity and mortality associated with neonatal jaundice due to G6PD deficiency.

MATERIALS AND METHODS

This hospital- and laboratory-based, cross-sectional descriptive study was done at 300-bedded Pyin Oo Lwin General Hospital and Pathology Research Division, Department of Medical Research (Pyin Oo Lwin Branch)**.** A total of 200 newborns which were delivered by any form of delivery were recruited by using random sampling procedure from January to March 2017**.** After getting an informed written consent from parents or caretakers, 2 ml of umbilical cord blood were collected into EDTA test tube for laboratory investigations. The specimens were labeled with newborn's code number, age, sex and date, and G6PD status was determined

using Brewer's test and G-Six kit methods (Reagents). If G6PD deficient occurred, blood was taken from a heel prick for measurement of serum bilirubin by Bilirubinometer at 300-bedded Pyin Oo Lwin General Hospital. Data entry was done by using Microsoft Office Excel and analysis was done by SPSS software 20.0 version. Frequency charts were defined and continuous variables' averages and standard errors (SE) were calculated. The prevalence rates were calculated with 95% confidence interval (95% CI).

Ethical consideration

The ethical approval was obtained from the Research Ethics Committee of the Department of Medical Research before the study was conducted.

RESULTS

A total of 200 newborns from which were delivered by any form of delivery in labor were enrolled as the participants. The participants were 103(58.5%) in female and 97(41.5%) in male, respectively. The modes of delivery in these subjects were 114(57.0%) in normal spontaneous vaginal delivery, 41(20.5%) in assisted delivery as vacuum and 45(22.5%) in lower (uterine) segment caesarean section (LSCS). Their birth weight ranged from 1.00-4.00 kg with a mean 2.92 ±0.59 kg.

Out of 200 newborns, 16(8.0%) newborns were G6PD deficient and 184(92.0%) were G6PD non-deficient by Brewer's method. Among them, 21(10.5%) neonates were G6PD deficient and 179(89.5%) neonates were G6PD non-deficient by G-Six kit method (Table 1).

Table. 1. Percentage distribution of G6PD deficiency by G-Six kit in newborns

	Number	Percent
G6PD deficient	21	10.5
G6PD non-deficient	179	89.5
Total	200	100

Out of 21 G6PD deficient cases, 17(80.95%) were males and 4 (19.05%) was females. The male to female ratio was 4.25:1 and male was statistically significant (p value <0.01) (Table 2).

Table 2. Sex distribution of G6PD deficiency by G-Six kit in newborns.

	Sex		Total
	Male n(%)	Female n(%)	
G6PD deficient	17(80.95)	4(19.05)	21(100)
G6PD non-deficient	80(44.69)	99(55.31)	179(100)
Total	97(48.5)	103(51.5)	200(100)

According to classification of G6PD deficiency (WHO 1989), there are five classes of G6PD deficiency exist based on magnitude of enzyme activity levels in red cells. In this study, moderate deficiency was 16 cases (76.19%) and mild deficiency was 5 cases (23.81%), respectively (Table 3).

Table 3. Distribution of G6PD activities in G6PD deficient newborns (according to classes of G6PD deficiency, WHO, 1989)

WHO classification	Number	Percent
Severe (less than 10%) activity	0	0
Moderate (10-60%) activity	16	76.19
Mild to normal (60-100%) activity	5	23.81
Total	21	100.00

Out of 21 (100%) G6PD deficient newborns, 9 cases (42.9%), 3 cases (14.3%), 4 cases (19%) and 5 cases (23.8%) were severe, moderate, mild hyperbilirubinaemia and normal bilirubin levels, respectively. There was found to be significant association between G6PD deficiency and serum bilirubin level (p value <0.05) (Table 4).

Table 4. The association between G6PD deficiency and total serum bilirubin level

Total serum bilirubin level	Moderate G6PD deficient	Mild G6PD deficient	Total
No hyperbilirubinaemia	2	3	5
Mild hyperbili rubinaemia	2	2	4
Moderate hyperbilirubinaemia	3	0	3
Severe hyperbilirubinaemia	9	0	9
Total	16	5	21

Overall, the Pearson correlation coefficient (r) was -0.662. Coefficient of determination (r^2) was 0.4382 and p value was 0.004. Therefore, there was moderate association between G6PD activity values and total serum bilirubin level which represented 43.8% of correlation between these values. There was statistically significant with p value <0.05.

DISCUSSION

In this study, G6PD deficiency was detected by using both the qualitative test (Brewer's method) and the quantitative enzyme assay test (G-Six kit method). The overall prevalence of G6PD deficiency was 8% (16/200) by Brewer's test whereas 10.5% (21/200) by G-Six kit method. However, only five partially deficient females were detected by G-Six kit method in this study. G6PD deficiency is sex-linked recessive disorder; males are more affected than female. Though sex-linked disorders are usually thought to affect males only, G6PD deficiency is also found in females, because of the high frequency of the gene and high incidence of consanguineous marriages. Not surprisingly, male is significantly higher than female using both tests in this study.

Brewer's method, one of the screening tests for G6PD deficiency, is an old method of qualitative analysis of G6PD deficiency with low specificity and sensitivity. More-over, it can detect only when the enzyme levels are 30% of the normal level.[11] The screening tests for G6PD deficiency can easily identify the defect among males but in females, by virtue of its X-linked inheritance, reliable results could only be obtained by quantitative assay.[12]

The clinical implication of using the enzyme assay method for screening of G6PD deficiency is that more high-risk infants could be detected early for close monitoring to prevent the development of severe hyperbilirubinaemia and kernicterus.[13] In 2009, El-Menshay *et al* found that the prevalence of G6PD deficiency in relation to neonatal hyperbilirubinaemia was about 30.2%. Similarly, according to 550-bedded MCH registry, the prevalence of G6PD deficiency associated with neonatal jaundice was about 30% which is detected by Brewer's method.

In the male Asian population, the incidence of G6PD deficiency is estimated to be 14% in Cambodia, 5.5% in South China, 2.6% in India, and less than 0.1% in Japan. It is rare among Native Americans.[14] In Asia, the deficiency prevalence ranges from 6.0% to 15.8%.[15, 16] In India, it is 10.5%[17] while in the Middle East the prevalence varies from 3% to 29%.[15, 16] Prevalence of G6PD deficiency in this study was concentrated predominantly among male children 17/97 cases (17.5%). Male sex was significantly correlated with G6PD deficiency among newborn studied (p=0.01). El-Menshay *et al* reported that out of the 16 G6PD deficient cases, 12(75%) were males and 4 cases (25%) were females with a male to female ratio 3:1 was similar to this study in which male to female ratio is 4:1 in G6PD deficient group.[9]

Current known and predicted prevalence of G6PD deficiency in Afghanistan, Bang-ladesh, Bhutan, India, Nepal, and Pakistan ranges from 0.7% to 10.7%, with regional "hot spots" exceeding 22%. Annually, 3.14 million infants are born at risk of G6PD deficiency in South East Asia countries and G6PD deficiency accounting for 33% of the global extreme hyperbilirubinemia burden, in contrast to 2.2% for those born in high-income nations.[20]

Prevalence of G6PD deficiency in this study was 21/200 cases (10.5%) in newborns and this finding is nearly consistent with the previous study in the Special Care Baby Unit and Post Natal Wards of Central Women's Hospital, Yangon, out of 520 healthy male term neonates, 53(10.2%) babies were G6PD deficient by Brewer's screening test. Of these 53 G6PD deficient subject, 45(85%) developed neonatal jaundice and 8(15%) did not develop jaundice.[21] In Myanmar, it was reported as

4-14% among various ethnic groups and 15-17% among populations residing in the malaria endemic areas.[22] A preliminary study on G6PD deficiency in neonatal jaundice at Yangon Children's Hospital showed 5.3% of neonates were G6PD deficient in 1994.[23] Moreover, the study on factors related to the severity of hyperbilirubinaemia in newborn babies admitted to Special Care Baby Unit (SCBU) of Mandalay Children's Hospital had been concluded that G6PD deficiency was one of the most frequent risk factors for hyperbilirubinemia.[8]

In the present study, out of 21 newborns 9 (42.9 %) were severe hyperbilirubinaemia, 3 (14.3%) were moderate hyperbilirubinaemia and 4 (19.0%) neonates were mild hyperbilirubinaemia. In correlation with severity of hyperbilirubinemia, there was a weak association between G6PD activity values and total serum bilirubin level. In this study, only the serum bilirubin level measured at the time of admission was analysed. A timed measurement of total serum bilirubin concentration is required to detect rise in serum bilirubin level. Thus, there was no enough evidence to determine severity of hyperbilirubinaemia in this study.

Thus, the use of G6PD activity values will also lead to substantial improvement in the identification and determining the severity of hyperbilirubinaemia. Indeed, the threatening consequence of G6PD deficiency is severe neonatal jaundice[9] and early detection of G6PD deficiency with appropriate management can prevent mental handicap and related developmental disabilities.[10] In this study, there was found to be significant association between G6PD activity values and serum bilirubin level. In other study in Turkey, Kilicdag *et al* showed that a significant correlation was found between the severity of hyperbilirubinaemia and G6PD activity in both males and females.[24]

Akhter *et al* studied G6PD status in neonatal jaundice and its relationship with severity of hyperbilirubinemia conducted on 90 males, term neonates with jaundice. They found that G6PD enzyme level was significantly lower in neonates with moderate and severe hyperbilirubinaemia than those with mild hyperbilirubinaemia. They concluded that severity of hyperbilirubinemia depends on degree of G6PD deficiency.[25] The prevalence of neonatal hyperbilirubinaemia is twice that of the general population in males who carry the defective gene and in homozygous females. It rarely occurs in heterozygous females.[26] This study proposed a fully quantitative G6PD screening kit employing the automated haemoglobin normalisation and a cut-off value of 6.4 U/g Hb. Any newborns with an activity below the cut-off value should be preventive measures should be taken.

The traditional use of Brewer's method is simple, cheap and widely available. The drawback of this method is that it can only detect all hemizygous males and homozygous females missing heterozygous females. In this study, high proportion of G6PD deficient neonates including partially deficient females so called heterozygous females were detected by enzyme assay method. Therefore, routine screening of G6PD activity values by enzyme assay method in hyperbilirubinaemia neonates especially in females is very important. Newborn screening for G6PD deficiency is not performed routinely in the United States, although it is done in countries with high disease prevalence. The World Health Organization recommends G6PD be introduced in populations with a male G6PD incidence of 3 to 5 percent or more.

In Myanmar, G6PD testing is not a routine test. The test is usually done when sign and symptom of haemolysis and neonatal jaundice occurs. The prevalence of G6PD deficiency differs greatly by some ethnic group in our country. In this study, the prevalence of G6PD deficiency is not low. International studies documented a benefit of neonatal G6PD screening in high risk population. Therefore, nationwide survey of G6PD deficiency should be done to get the evidence data of G6PD deficient ethnic group.

Recommendations

- Screening for G6PD deficiency in routine clinical practice should be considered in high-risk groups.
- Health education is needed to ensure a greater awareness of G6PD.
- Future research is needed to evaluate mutation on G6PD deficiency by molecular methods

Competing interests

The authors declare that they have no competing interests.

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