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Glucose-6-Phosphate Dehydrogenase Deficiency among Children Attending the Emergency Department of Yankin Children's Hospital, Yangon

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Yankin Children's Hospital is one of the tertiary children hospitals in Myanmar, where some oxidative medications are commonly used in the management of illnesses. Paediatrician's awareness of G6PD status in this population is very important for effective management and prevention of complications in G6PD deficient children. This preliminary study aims to determine the prevalence of G6PD deficiency according to WHO classification among children seeking medical care at Emergency Department of Yankin Children's Hospital (YKCH). It was a cross-sectional descriptive study on 124 children, aged 1 month to 13 years. G6PD enzyme activity was determined by spectrophotometric assay within 24 hours of sample collection. Randox G6PD quantitative *in vitro* test kit (Randox Laboratories, Crumlin, UK) was used and G6PD activity was calculated as unit per gram (U/g) of haemoglobin (Hb). For classification of G6PD deficiency, 10% and 60% level of normal enzyme activities were calculated according to the suggestion by World Health Organization (WHO); G6PD activity <10% was defined as severe deficiency and 10-60% was defined as moderate deficiency. According to WHO classification, 18.5% (23/124) of children in this study was classified as G6PD deficient, with 3.2% severe deficiency and 15.3% moderate deficiency. The prevalence of G6PD deficiency in Myanmar children is higher than the previous reported prevalence if quantitative spectrophotometric method is used for diagnosis, detecting more individuals with moderate deficiency. The high prevalence of G6PD deficiency in this study warrants for the need to do neonatal screening to avoid the potentially fatal complications of this disease.

Keywords: Glucose-6-phosphate dehydrogenase, G6PD, Children

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy in human, affecting approximately 400 million people[1] with the highest prevalence in Africa, Asia and Mediterranean countries.[1] The burden of this disease is also significant in Myanmar, with the adult prevalence of 11.1% in male and 4.2% in female.[2] Approximately 10% of newborn babies delivered at teaching hospitals in Yangon were G6PD deficient.[3, 4] This enzyme deficiency also contributed to 17%-36% of phototherapy needed neonatal jaundice in neonatal units of children hospitals in this country.[5] Although there is a high prevalence of this enzyme deficiency in Myanmar, most people are not aware of their G6PD status because screening of the disease is not a routine practice yet and most of the affected persons are asymptomatic. However, if G6PD deficient people are exposed to oxidative stress, acute intravascular hemolysis may occur leading to heart failure and even death, if there is no timely intervention.

Most of the reported prevalence of G6PD deficiency in Myanmar are the results by semi-quantitative assays[2-5] though definitive diagnosis of G6PD deficiency is by quantitation of red cell enzyme activity.[6] However, there is no internationally accepted threshold for G6PD deficiency[7] and levels of enzyme activity may

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differ with different G6PD variants in different populations.[8] There is paucity of local data in Myanmar children regarding quantitative G6PD enzyme activity.

YKCH is one of the tertiary children hospitals in Myanmar, where some oxidative medications like sulfonamides (eg. sulfamethoxazole) and non-sulfa antibiotics (eg. isoniazid) are commonly used in the management of illnesses. Although the paediatricians are aware of the importance of G6PD deficiency, data regarding G6PD status in this population is lacking.

These data are very important for effective management and prevention of complications in G6PD deficient children. This study aims to define the cut-off values of G6PD enzyme activity by spectrophotometric assay and to determine the prevalence of G6PD deficiency according to quantitative cut-off values in children seeking medical care at Emergency Department of YKCH, as a preliminary study.

MATERIALS AND METHODS

By using the sample size calculation formula for proportion ($N=Z^2pq/d^2$), with 9% prevalence of G6PD deficiency in children,[9] 5% precision of margin of error and 95% confidence interval, the required sample size was 125 cases.

A cross-sectional descriptive study included children with aged 1 month to 13 years visiting the Emergency Department of YKCH. A total of 131 children were screened to exclude acute haemolysis (by history and clinical examination of sudden onset of pallor, jaundice and dark colour urine), and history of blood transfusion within 1 month duration. Recent dengue infection was also screened in these children by testing with SD BIOLINE Dengue Duo rapid diagnostic test for dengue (Standard Diagnostics, Korea). Five cases that could have acute hemolysis and 2 cases with recent dengue infection were excluded from the study, leaving 124 children in data analysis.

Approximately 3 milliliters of blood were taken from each child collected in ethylene diamine tetraacetic acid (EDTA) anticoagulated vacutainer tubes. Hemoglobin (Hb) level was examined by coulter counter. Anaemia was defined if Hb level is <11 g/dl in under-5 children and <11.5 g/dl in children who were older than 5 years, respectively.[10] G6PD enzyme activity was determined by spectrophotometric assay within 24 hours of sample collection at the National Health Laboratory. Randox G6PD quantitative *in vitro* test kit (Randox Laboratories, Crumlin, UK) was used and G6PD activity was calculated as unit per gram (U/g) of haemoglobin (Hb). Whole blood (0.2 ml) was washed with 2 ml aliquot of NaCl solution, centrifuged for 10 minutes at 3000 rpm (repeated three times). The supernatant was used for the enzyme assay within 2 hours of preparation and photometric measurement of the kinetic reaction was done at 340 nm. For classification of G6PD activity, median level of G6PD activity in boys was calculated and all results with <10% of G6PD activity was excluded. The adjusted median values on the remaining samples of boys were calculated again and defined the resulting G6PD activity as 100%. Based on this reference value, G6PD cut-off activities for G6PD deficiency at 10% and 60% were calculated.[11]

According to World Health Organization (WHO) criteria, G6PD activity <10% was defined as severe deficiency and 10-60% was defined as moderate deficiency.

Statistical analysis

Data were analyzed by using Statistical Package for the Social Sciences (SPSS) version 16.0. Descriptive analysis on frequency and percentages of gender and G6PD deficiency was reported. Age was expressed as median (IQR). Comparisons of baseline characteristics of children for G6PD status were made by Chi square test. P value <0.05 was denoted as statistically significant.

RESULTS

The study included 124 children, in which 69(55.6%) were boys and 55(44.4%) were girls, with the median age of 3.7 years (IQR; 1.8-6.3 years). The median and range of G6PD activity of children attending OPD of YKCH were described with different sexes in Table 1.

Table 1. G6PD activity profile in children attending OPD of Yankin Children's Hospital

Values	Total (n=124)	Female (n=55)	Male (n=69)	Adjusted male(n=66)
Median (U/g Hb)	5.4	5.4	5.3	5.4
Range	0.2-11.6	0.2-11.6	0.2-10.4	2.3-10.4

The median G6PD activity in 69 boys was 5.3 U/g Hb. The adjusted male median value of G6PD activity was determined by excluding 3 male samples with <10% of enzyme activity from the derived value. The adjusted male median activity in 66 boys was 5.4 U/g Hb (2.3-10.4) (Table 1). Based on this value, G6PD activity <0.54 U/g Hb was proposed to define as severe deficiency (1-10% of residual activity) and 0.54-3.24 U/g Hb was proposed to define as moderate deficiency (10-60% of residual activity).

Table 2. Proportion of participants with G6PD deficiency according to residual G6PD enzyme activity cut-off values

	Percent G6PD cut-off activity		Total number of G6PD deficiency (%) n(%)
	<10% (<0.54 U/g Hb) n(%)	10-60% (0.54-3.24 U/g Hb) n(%)	
Male (n=69)	3(4.3)	10(14.5)	13(18.8)
Female (n=55)	1(1.8)	9(16.4)	10(18.2)
Total (n=124)	4(3.2)	19(15.3)	23(18.5)

Proportion of participants with residual G6PD enzyme activity and cut-off activity was demonstrated in Table 2. A total of 18.5% (23/124) of children were classified as G6PD deficient. According to WHO classification, 4 children (3.2%) were in severely deficient group (1-10% residual activity) and 19 (15.3%) were in moderately deficient group (10-60% residual activity) based on the enzyme activity of children in this study.

Table 3. Participants with G6PD deficiency related to baseline characteristics

Baseline characteristics	G6PD deficient n(%)	p value
Gender		
Male (n=69)	13(18.8%)	0.9
Female (n=55)	10(18.2%)	
Age		
<5 year (n=76)	14(18.4)	0.9
5-13 year (n=48)	9(18.8)	
Nutritional status		
Malnutrition (n=25)	3(12.0)	0.3
Normal (n=99)	20(20.2)	
Haemoglobin status		
Anaemia (n=33)	15(21.4)	0.3
Normal (n=98)	8(14.8)	

The participants with G6PD deficiency related to baseline characteristics of children were described in Table 3. G6PD deficiency was not significantly associated with gender, age, nutritional status or hemoglobin status.

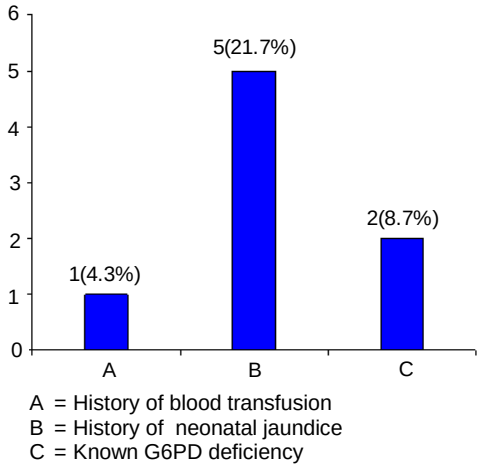

Fig. 1. Past history in 23 G6PD deficient children

The past history of G6PD deficient children noted that only 8.7% (2/23) had known G6PD deficiency status previously, 21.7% (5/23) had history of neonatal jaundice and 4.3% (1/23) had history of blood transfusion (Fig. 1).

DISCUSSION

There are no previously proposed quantitative values for G6PD deficiency on Myanmar population. It is crucial to define quantitative G6PD activity cut-off values in this country because the criteria for other populations are not universally applicable and may not be suitable for Myanmar people. In this study, the cut-off values for G6PD deficiency were proposed based on the population of children visiting Emergency Department of YKCH.

Studies over different populations suggested different criteria for G6PD cut-off activities. The suggested 100% G6PD activity of this study is slightly higher than the mean G6PD activity 4.49±13.8 U/g Hb of 201 children (1.5-13.8 years age group) visiting day-care centers of the city of Sao Paulo using Test Combination G6PDH kit.[12] It was also higher than the mean G6PD activity of 4.1±2.48 U/g Hb in 933 Nigerian neonates by using the same Randox kit.[13] However, the mean activity in this study was much lower than the mean values in Malaysian studies; 10.18±3.36 U/g Hb of 236 Malaysian children with the same age group using OSMMR2000-D kit[14] and 14.8 U/g Hb of Malaysian neonates using the same Randox kit.[15] The higher value in Malaysian children with the same age can be partly explained that all the participants were G6PD non-deficient children, already screened by fluorescent spot test.

The proposed cut-off values (<0.54 U/g Hb as severe deficiency and 0.54-3.24 U/g Hb as moderate G6PD deficiency) in this study are also lower than those in some adult studies. Recent study in 1000 Myanmar adults reported that median G6PD activity in normal male population was 8.28 IU/g Hb by Trinity Biotech G6PD assay.[16] LaRue, *et al.* define 0.7 IU/g Hb and 4.3 IU/g Hb as <10% and <60% enzyme activity on 214 people using Trinity Biotech G6PD assay.[17] Nantakomol, *et al.* proposed 0.95 IU/g Hb and 5.7 IU/g Hb as cut-off points for severe and partial deficiency based on the study involving 295 Thai adults using G6PD kit from BIOLABO.[8] Espino, *et al.* demonstrated the cut-off values for severe and partial deficiency as 0.98 and 5.88 IU/g Hb in 621 Philippines by using Trinity Biotech G6PD kit.[18]

Apart from age group involved in the studies, the differences in quantitative values of G6PD activity may also be due to the different comercial kits used. LaRule, *et al.* reported that reference values vary widely depending on the reference tests used.[17] In their study, the mean activity value measured by R & D test (10.33 U/g Hb) was higher than the Trinity test (7.17 U/g Hb) and the differences between the two tests were statistically significant.[17] Even using the same test kit, enzyme activity can also depend on specimen handling and reaction condition like assay temperature. Care must be taken in each step of sample collection, transport and laboratory testing. The reference values described in comercial kits may not be acceptable in every population to define G6PD deficiency. It is recommended in the manual of the Randox G6PD kit that each laboratory should establish its own reference range to reflect the age, sex, diet and geographical location of the population. Some prevalence studies using Randox kit used cut-off value of <2.9 U/g Hb for complete G6PD deficiency as Ainoon, *et al.* proposed.[15] This criterion may not be suitable for all populations, because various ethnic groups have different G6PD variants and hence levels of enzyme activity may not be the same.

Based on the cut-off values of <3.24 U/g Hb for diagnosis of G6PD deficiency, 18.5% of the study population have G6PD deficiency, which is higher than the previously reported values of 9-10.5% in children by using some semi-quantitative assays. A study which screened 100 healthy children aged 5-12 years at a malaria endemic area by using Congent test reported that 9% were G6PD deficient.[9] Nearly the same prevalence of G6PD deficiency (10.5% and 10.2%, respectively, by Brewer's test) was reported in newborn babies delivered at North Okkalapa General Hospital and Central Women's Hospital.[3, 4] In the study conducted at Central Women's Hospital, the same children were also screened by florescent spot test and the prevalence was reported to be 9%.[3] Studies reported that semi-quantitative assays could detect only the cases with the residual red cell activity of less than 20-30% of normal, missing significant proportions with moderate enzyme deficiency.[19] Higher prevalence of G6PD deficiency in this study may be because of detecting more cases with moderate enzyme deficiency like heterozygous females and males with class III G6PD variants.

In this study, history of neonatal jaundice and blood transfusion were present only in 21.7% and 4.3% of cases with G6PD deficiency, and only 8.7% of G6PD deficient children had already known their disease status. Baseline characteristics like age and sex, and general conditions like nutritional status and anaemia cannot differentiate G6PD deficient children from normal ones. This means that majority of G6PD deficient population does not know their disease situation and clinicians cannot differentiate the children with G6PD deficiency from non-deficient ones based on their general condition; which can lead to increased susceptibility to hemolysis when given some oxidant medications. Although WHO recommends the population screening of G6PD deficiency in regions where the male prevalence is 3-5% or more,[7] newborn screening still cannot be performed routinely in Myanmar.

Conclusion

The prevalence of G6PD deficiency in Myanmar children is higher than the previous reported prevalence if the quantitative spectrophotometry is used for diagnosis. This warrants for the need to do neonatal screening to avoid the potentially fatal complications of this disease. As a preliminary study, this study also proposed the enzyme activity level of <0.54 U/g Hb as severe deficiency and 0.54-3.24 U/g Hb as moderate G6PD deficiency based on the population of children visiting Emergency Department of YKCH by using Randox spectrophotometry assay. Studies with larger sample size in different age groups and with

other commonly used comercial assay kits should be done to determine the normal reference range for G6PD activity in Myanmar population.

Competing interests

The authors declare that they have no competing interests.

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