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Glucose 6 phosphate dehydrogenase levels in babies delivered at the University of Ilorin teaching hospital.

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Abstract *Background:* Glucose-6-phosphate dehydrogenase deficiency, an X-linked recessive disorder, is the most common enzymopathy producing disease in humans.It is known to cause severe neonatal hyperbilirubinaemia.
Aims and Objectives: To determine G6PD levels in babies delivered at the University of Ilorin Teaching Hospital with a view to determining the prevalence of G6PD deficiency.
Methods: Samples of cord blood were collected at delivery, from 933 babies who met set criteria. Blood was assayed for G6PD levels using a quantitative in vitro test (RANDOX©).
Results: A total of 348 (37.3%) of the 933 tested subjects had G6PD deficiency with enzyme activity of ≤ 2.8U/gHb. Glucose 6 Phosphate Dehydrogenase levels in female babies with normal enzyme levels were significantly higher than in male babies with normal enzyme levels (5.72 ± 2.45 U/gHb versus 4.99 ± 2.3 U/gHb, p = 0.002). Enzyme levels in babies with G6PD deficiency was comparable in both males and females (2.05 ± 0.60 u/gHb in females and 2.1 ± 0.66 U/gHb in males, p = 0.66). The prevalence of G6PD deficiency was comparable among males and females (p = 0.81, χ^2 = 0.06, RR = 1.02, CI = 0.90 < RR < 1.15, OR=1.04).
Conclusion: There is a high prevalence of G6PD deficiency in babies delivered at the University of Ilorin Teaching Hospital, and the enzyme deficiency appears to occur equally among the sexes.

Key words: Glucose-6-phosphate, neonates, cord blood

Introduction

Glucose-6-phosphate dehydrogenase (G-6-PD) is an enzyme in the hexose monophosphate shunt that catalyses the oxidation of glucose-6-phoshate to 6-phosphogluconate. This is the rate limiting step of the pathway. Concomitantly, Nicotinamide Adenide Dinucleotide Phosphate ($NADP^+$) is reduced to NADPH.[1] The NADPH, a required co-factor in many biosynthetic reactions, maintains glutathione in its reduced form. Reduced glutathione acts as a scavenger for free radicals, and thus helps reduce oxidized haemoglobin to free haemoglobin; otherwise oxidized haemoglobin will precipitate as Heinze bodies. The message of haemolysis has NOT been sent by this presentation. While many other body cells have other mechanisms of generating NADPH, the red blood cells rely completely on G-6-PD activity because it is the only source of NADPH that protects the cell against oxidative stress.[2]

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is the most common disease producing enzymopathy in humans. It is inherited as an X linked recessive disorder. It affects about 400 million people worldwide.[3] The highest prevalence rates occur in persons of African, Asian, Mediterranean or Semitic descent.[4] Specific prevalence rates worldwide range from 3.9% in India[5] 12.8% in the

USA[6] and 50% in the Middle East.[7] In Nigeria, an estimated 21% of the male population is said to have G-6-PD deficiency.[8] In neonates, the prevalence among jaundiced babies range from 30.9% - 50%in Nigeria.[9-12]

G-6-PD deficiency causes a clinical spectrum of illness which includes a purely asymptomatic state, acute haemolytic episodes (from drugs , infections, ingestion of fava beans, diabetes mellitus), chronic haemolysis (hereditary non-spherocytic haemolytic anaemia), and neonatal jaundice.[13]

Few studies have been conducted, in this part of the world in this part of the world, to determine the G6PD status of the newborn prior to the onset of jaundice. This study was aimed to determine the G6PD enzyme level in newborn babies prior to the onset of jaundice, and to relate these enzyme levels to the baby's gender/sex.

Methods

Patients and Treatment

This cross sectional prospective study was performed at the maternity unit of the University of Ilorin Teaching Hospital, which is located in the North Central region of Nigeria. Ethical approval was obtained from the institutions' Ethical Review Committee of the institution. Average yearly deliveries range from 4500 5000 babies, with virtually all babies being of West African descent.

Alternately delivered newborn babies whose mothers gave consent were recruited into the study. Exclusion criteria included babies whose gestational age was greater than 42 weeks, babies with severe birth asphyxia, and those with congenital abnormalities.

Blood from the placental end of the cord was drawn and G6PD levels were assayed using an *in vitro* diagnostic kit manufactured by RANDOX© Laboratories Limited (Ardmore Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT 294 QY.

Laboratory Methods

For G6PD assay, 2ml of blood drawn from the placental end of the cord was collected into EDTA containing bottles after delivery. Enzyme level was assayed using a quantitative in vitro test (RANDOX©). Its principle is based on reduction of NADP+ by G6PD present in red blood cells. The NADPH generated fluoresces under UV light at a wave length of 340nm. Enzyme activity is determined by the rate of absorbance change. Red blood cell G6PD value of $\geq$ 2.9 U/gHb was regarded as normal.[14]

Data Analysis

This was done using EPI-info version 6 software.[15] Association between categorical variables were tested using Chi-square test. Relationship between a continuous variable and dependent variable were tested using the Student t test or ANOVA as appropriate.

For all statistical analysis, $p < 0.05$ was considered significant.

Results

Study Population

The study was conducted over a 10 month period during which 4591 babies were delivered at the University of Ilorin Teaching Hospital. They comprised 2525 males and 2066 females with a male to female ratio of 1.1:1. The gestational age of the babies ranged from 26 weeks to 44 weeks, with a mean GA of 39 $\pm$ 3.9 weeks. Birth weight ranged from 7005450g with a mean of 3892 $\pm$ 1148g. A total of 933 babies had samples for G6PD assay collected during this study.

Table 1 shows the baseline characteristics of study subjects. There were 903 (96.8%) singleton deliveries and 15 (3.2%) sets of twins. Babies delivered at term constituted 79.9% of the study population, while preterm babies made up 20.1% of the study population. The male to female ratio was 1.3:1.

Table1: Baseline Characteristics of study subjects

Variables	Enrolled (%)
Type of Delivery	
Singleton	903 (96.8)
Twins	15 (3.2)
Gestational age (GA)	
Preterm	187 (20.1%)
Term	764 (79.9%)
Gender	
Male	514 (55.1)
Female	419 (44.9)
Male : Female	1.3:1

Figures in brackets are percentages of 933

Table 2 shows the distribution according to mean birth weight and gestational age. The gestational age (GA) of the babies studied ranged from 26 to 42 weeks with a mean GA of 38 ± 2.7 weeks. Birth weights ranged from 800 - 5050g, with a mean of 2866.9 ± 710g grammes.

Table 2: Mean birth weight in relation to gestational age

Gestational Age (weeks)	Number (%)	Mean birth weight (gm)
26 – 29	18 (1.9)	1107.5 ± 370
30 – 33	68 (7.3)	1565.2 + 252
34 – 36	101 (10.8)	2282.4 ± 476
37 – 40	653 (69.9)	3099.7 ± 509
41 – 42	93 (10)	3217.3 ± 626
Total	933	2866.9 ± 710

Glucose -6-Phosphate Dehydrogenase Levels in the Subjects

Glucose-6-Phosphate Dehydrogenase levels that were ≥2.9U/gHb were regarded as normal, while values ≤2.8U/gHb were regarded as deficient.[14]

Stratification of G6PD levels into various ranges

Table 3: Relative proportion of G6PD values

G6PD values (U/gHb)	Number (t=933)
= 1	26 (2.8%)
2	86 (9.2%)
2 -2.8	236 (25.3%)
2.9 - 7.2	457 (49%)
7.3 - 10	94 (10.1%)
>10	34 (3.6%)

Three hundred and forty-eight babies (37.3% of 933) were found to be G6PD deficient, thereby giving a hospital based prevalence of 37.3%. They comprised 194 males (37.7% of male population) males and 154 females (36.8% of female population) females (p = 0.81, χ^2 = 0.06 RR = 1.02, CI=0.90<RR<1.15, OR=1.04).

The overall mean G6PD value was 4.1 ± 2.48 U/gHb (range 0.87 13.0 U/gHb). Among those with normal G6PD values, males had a mean G6PD value of 4.99 ± 2.3 U/gHb and females 5.7 ± 2.45 U/gHb. Thus, normal females had a mean G6PD value that was significantly higher than that of normal males (p = 0.002).

Enzyme levels in babies with G6PD deficiency was about equal in both sexes (2.1 ± 0.66 U/gHb in males And 2.05 ± 0.60 U/gHb in females, p = 0.66). (Table 4)

Table 4: Glucose-6-Phosphate Dehydrogenase levels in normal and deficient neonates according to sex

Classification	Mean	(N)	F	P
Normal				
Male	4.99 ± 2.3 U/gHb	320		
Female	5.7 ± 2.45 U/gHb	265	9.39	0.002
Deficient				
Male	2.1 ± 0.66 U/gHb	194		
Female	2.05 ± 0.60 U/gHb	154	0.32	0.6

	Male (%)	Female (%)	χ^2	P
Normal	320	265		
Deficient	194 (37.7)	154 (36.8)	0.06	0.81

Discussion

The overall prevalence of G6PD deficiency as shown in the study was 37.3% in the neonatal population. In other words, about four out of every 10 babies born at the teaching hospital was G6PD deficient. The method used in enzyme assay in this study was quantitative enzyme assay, rather than qualitative (fluorescent spot). The fluorescence spot test is based on the visual evaluation of fluoresced reduced NADPH when activated by ultraviolet light so that, the sample is considered G6PD enzyme "deficient" when they do not fluorescence, and "normal" when they fluorescence.[14] Studies have shown that false negative results may occur, with the use of the fluorescent spot test, in heterozygote females and in homozygote males following an acute haemolysis.[14,16,17] In one study involving known female heterozygotes, G6PD deficiency was diagnosed in 53% of these females by the use of enzyme assay, but in only 7.5% of these females with the use of the fluorescence spot test.[14] This (florescent spot test) was the method used in enzyme assay in studies done in Nigeria thus, the prevalence rate in this study is at a variance with rates determined by other workers viz, 20.5%,[18] 20.6%,[19] and 35.3%.[20]

Additionally, the babies recruited into these studies already had jaundice.[18,19,20] Enzyme levels assayed during an acute haemolytic event will likely

demonstrate enzyme activity in reticulocytes and neocytes.[21] In the enzyme variant responsible for deficiency in the West African sub-region, GdA$^-$,[22] enzyme activity wanes as the cells age so that neocytes have near normal enzyme activity.[23] Thus, earlier studies conducted in this region would have underestimated the level of enzyme deficiency in neonates.[15]

Studies, in this environment, where G6PD enzyme was assayed prior to the onset of jaundice are few, and hence a relative dearth of information on either the incidence or prevalence rate of the enzyme deficiency.[24] In one study where enzyme activity was assayed from cord blood samples,[24] the prevalence rate of G6PD deficiency did not appear to be the aim of the researchers. Other studies in which G6PD enzyme assays were determined at birth reveal prevalence rates of 12.8%[6] in the African-American population in the US, 7.6%[14] in Malaysia, and 3.2%[25] in Iran. The link between G6PD deficiency and malaria is well documented,[15,19,22] and with malaria being holoendemic in Nigeria a higher prevalence of the enzyme deficiency is to be expected.[26] Luzzatto[27] in his work discovered that 22% of Nigerian adult males, and 3 - 4% of female homzygotes were G6PD deficient.

A similar proportion of females and males, in this study, were found to be enzyme deficient ($\chi^2 = 0.06$, p = 0.81, RR = 1.02, CI=0.90<RR<1.15, OR=1.04). In another study done on neonates with jaundice, using a quantitative assessment of enzyme activity, 58% of females and 50.3% of males in the study population were found to be G6PD deficient.[28] The cut off for enzyme deficiency used in that study were levels that were 40% of normal adult values (8.83 ± 1.59eU/gHb). This finding is contrary to what would be expected of an X-linked disorder. It would naturally be expected that the enzyme deficiency would occur more in males because they carry only one X chromosome. The females are however in the unique position to have 3 genotypes: normal homozygous, heterozygous and deficient homozygous.[29] Because of random X chromosome inactivation by Lyonization[13] the heterozygote female has two red cell populations: one G6PD deficient, and the other G6PD normal. In most instances, the heterozygote female still has normal enzyme activity but, the total G6PD activity of the heterozygote female can range from near normal to near deficient.[13,15] It has also been postulated that in geographic areas where G6PD deficiency is very common, female newborns might be homozygous for the trait, thus behaving like the hemizygous G6PD deficient male newborn.[15] A DNA analysis, which would have served as a tie-breaker, was beyond the financial scope of this study.

Overall, enzyme levels in normal females were clearly higher than the levels in normal males. This finding can, however, not be explained. The gene for the G6PD enzyme is located on the X chromosome and so, because the female is doubly endowed, she should demonstrate higher enzyme levels. However, because of the influence of Lyonization[13] enzyme levels in the female should not be appreciably higher than in males. This will serve as grounds for further research.

In view of the high prevalence of G6PD deficiency in this region, newborn screening programmes should be instituted to aid in the early diagnosis and preemptive care of babies with this condition.
The major limitations of this study were our inability to carry out DNA analysis on the sample drawn from female neonates (financial and technological constraints), and the low number of early preterm neonates recruited.

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