

NEW BORN SCREENING FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN ORISSA, IMS & SUM HOSPITAL: A QUANTITATIVE ASSAY

ABSTRACT

Objectives: To determine the incidence of G6PD deficiency in new born admitted to IMS & SUM HOSPITAL, Bhubaneswar. An objective of the study was to estimate the incidence of G6PD deficiency among new-borns and its association with different socio-demographic, clinical and gestational characteristics.

Methods: A total of 191 new born blood samples were screened for the quantitative measurement of G6PD activity by enzymatic colorimetric assay by a commercial kit Kinetic Method (G-SIX KIT). Any neonate with a value < 6.4 U/gHb was considered G6PD deficient.

Results: Of the 191 new-borns (120 males, 71 females) screened, 32 neonates were found to have G6PD deficiency (24 males, 8 females). The overall incidence of G6PD deficiency was 16.75%. Frequency in male population was 20% (24 out of 120 male neonates) and in female population was 11.26% (8 out of 71 female neonates).

Keywords: Glucose-6-phosphate dehydrogenase deficiency, , Haemolytic crisis, Gestational age

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is among the most common inherited haemolytic disorders found among humans, affecting around 400 million people worldwide^[1]. In contrast with the high prevalence of this disease in many populations around the world, new born screening for G6PD deficiency has been implemented and incorporated into the screening program in several non-western countries, such as in the Middle East, Eastern Europe and Southeast Asia⁽³⁾. In India, out of 13 biochemical variants of G6PD being reported so far, G6PD Mediterranean is most common in the caste groups, whereas G6PD Orissa is most prevalent in the tribal of India. The 3rd most common variant seen in India is G6PD Kerala-Kalyan⁽⁶⁾. Kaeda et al found that G6PD Orissa is most common G6PD variation in tribal populations and not in the urban population. This distinct variation in the G6PD variants between the tribal and caste populations may be due to differential selection or due to different evolutionary histories of these two groups⁽⁷⁾. G6PD deficiency is an X-linked disorder mostly affects African, Mediterranean and far-eastern populations.^(2,3) G6PD is the first enzyme of the pentose phosphate pathway and catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone, with the concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form (NADPH). NADPH protects cells from oxidative stress. Haemolytic anaemia and neonatal jaundice are the two major conditions associated with G6PD deficiency. Haemolytic anaemia results from stress factors such as certain oxidative drugs, infections or fava beans.⁽⁴⁾ G6PD deficiency is also known as 'favism', is a condition of acute haemolytic anaemia after the consumption of broad beans (*Vicia faba*) – a common food found in Iran⁽⁵⁾. Severe neonatal jaundice may be a manifestation during neonatal period, which in few cases cause severe neurological complications and even death in some populations.^(6,7) The exact mechanism of jaundice in G6PD deficient neonates is not completely known, haemolysis alone does not seem to contribute as much as impaired bilirubin conjugation and clearance by liver⁽⁸⁾

Neonatal screening for G6PD deficiency has found to have beneficial in many countries with high disease prevalence⁽²¹⁾. The World Health Organization recommends screening all new-borns in populations with a prevalence of 3–5% or more in males⁽¹¹⁾. In malaria endemic and non endemic areas G6PD deficiency has a prevalence of 5% to 25% and 5% respectively⁽⁹⁾. Malaria is the important known selective force in the recent history of mankind. A number of polymorphisms associated with erythrocyte cell surface oligoproteins (blood groups), globin genes (HbS, HbC, HbE, thalassemia, oxidative stress (G6PD deficiency), cytoadherence and immune system have

been associated with protection against malaria. The impact of malaria on human genetic polymorphisms has been reviewed by Miller⁽¹⁰⁾ and Kwiatkowski⁽⁹⁾.

MATERIALS AND METHODS

This is a cross-sectional and prospective study consists of all inborn and outborn babies admitted to the Hospital Three cc EDTA(ethylondiamentetracetic acid) cord blood samples collected for each neonate from the Labour Room/NICU/SCNU and all samples were stored at 2~6⁰ c for routine screening of G6PD deficiency by Kinetic Method(G-SIX KIT). As routinely practised in this hospital, results of all G6PD screening tests will be collected within 24 hours of receiving the specimen and mothers of babies diagnosed as G6PD deficient will be explained by the attending Paediatrician regarding the problems related to the disorder and advised to have the babies kept in the Hospital for a minimum of five days to observe for the development of clinical jaundice. The neonates' sex, weight, height, head circumferences, gestational age, maternal age and parents' consanguinity were recorded on questionnaires and transferred to coding sheets in a computer database. All the babies were followed up for a period of at least 3 days. Those who developed jaundice were followed up for at least 1 week in the neonatology ward, because these babies were transferred to this unit for treatment of jaundice. By using the standard method of estimation of bilirubin (van den Bergh reaction) in the laboratory of neonatology unit, the incidence of severe jaundice (serum total bilirubin > 15 mg/dl) in them was documented. Analysis of data was done by standard methods of descriptive statistic

RESULTS

Of 230 subjects enrolled in the study, 39 (27 males and 12 females) were excluded, either because of clotted or insufficient blood samples, or because the parents did not give consent for sampling. Therefore, of the 191 new-borns 120 [52.3%] males 71 [47.7%] females) screened over a period of nearly one year, 32 neonates were found to have G6PD deficient (24 males, 8 females). The overall incidence of G6PD deficiency was 16.75%. Frequency in male population was 20% (24 neonates of 120 male neonates) and in female population was 11.26 (8 neonates of 71 female neonates). The female: male ratio for G6PD deficiency was 1:2.5. There was no difference in the prevalence of parental consanguinity among female G6PD deficient and female normal infants. Also, no statistical gestational age difference was found between the two groups

Criteria for some of the variables are: Blood of individuals having G6PD activity below 6.4 U/g Hb of Hb (Normal 6.5-20 U/g Hb of Hb) were considered G6PD deficient^[6]. Preterm babies had been defined as those neonate having gestational age of < 37 weeks, and low birth weight (LBW) babies were those whose birth weight was < 2.5 kg⁽⁷⁾. Total serum bilirubin > 15 mg/dl⁽⁷⁾ was considered as having significant jaundice.

Distribution of cases according to different Socio-demographic factor of new born

One hundred & thirty eight new-borns (72.25%; n=191) in this study were term (GA >37wk) and 53 (27.74%) were preterm (GA <37wk). twenty four (17.39% n=138) term & eight (15.09% n=53) preterm babies were G6PD deficient. This difference in frequency of G6PD deficiency among term and preterm babies was not statistically significant (p >0.05) (Table-1)

There was gender difference in enzyme activity when the data of all new-born infants (G6PD deficient and normal infants) were analysed together (P =0.1184). Religion, ethnicity, consanguinity, birth weight & severe jaundice at birth does not show any statistically significant (p >0.05) for G6PD deficient (Table-1).



		Total case	G6pd normal	G6PD deficiency	Chi square	P value
Sex	Male	120	96	24	2.439	0.1184
	Female	71	63	8		
Religion	Hindu	178	47	31	0.821	0.3648
	Muslim	10	9	1		
	Christian	3	3	0		
Ethnicity	odhisa	183	52	31	0.108	0.7420
	Nonodhisa	8	7	1		
Consanguinity	Yes	8	7	1	0.108	0.7420
	No	183	160	31		
Gestational age	Term	138	114	24	0.145	0.7035
	Preterm	53	45	8		
Birth weight	Lbw	60	52	10	0.000	0.9826
	Normal weight	131	109	22		
Severe jaundice at birth	Yes	61	49	12	0.547	0.4595
	No	130	110	20		

Lbw- Low birth weight

Table 1: Relation of glucose-6-phosphate dehydrogenase deficiency with different Socio-demographic factor of new born (N =191)

DISCUSSION

Our data revealing the prevalence of G6PD is relatively high in the east odisa (16.67%) in general population and 20% and 11.26% in male and female population, respectively. There has been no precise previous information about the incidence of G6PD deficiency in neonates of this part of odisa. Several studies have reported incidence in other parts of Orissa, and the prevalence varies markedly among different communities. These studies gave a high rate of 10%–16.67% in the eastern part (khurdha, nayagarah, puri & Cuttack dist.). The frequency of G6PD deficiency gene in various primitive tribal population of Orissa was very high (Munda 15.9%, Kharia 14.2%, Bhuyan 12.9%, Kolha 9.8%, Bhatari 9.5%, Santal 9.0% and Saura 7.7%^(11,28)). In a study conducted by the RMRC, Bhubaneswar, the prevalence of G6PD deficiency was found to be 0.36% in Bondo, 1.6% in Didayi, 7.3% in Juanga and 4.8% in Kutia Kondha primitive tribes. An in depth molecular analysis amongst the G6PD deficient tribal subjects including Juanga primitive tribes of Keonjhar district of Orissa revealed the presence of a new variant of G6PD known as G6PD Orissa^(11,28). G6pd has been studied in two tribal population Kissan tribes of Orissa & Kannikar tribes of nedumungad taluk, Trivandrum, Kerala. The percentage frequency of G6PD deficiency are 14.13 & 5.5 respectively⁽²⁸⁾.

A study conducted on the North-Eastern India i.e. Mizo population of Mizoram showed that out of the 490 study subjects, 17.5% were found to be G6PD deficient⁽¹⁴⁾. These differences may be due to different genetic types of G6PD in different caste and ethnic groups in India. The distribution of G6PD deficiency in Indian population is population specific. The frequency is higher among the tribals than the caste populations⁽¹⁵⁾. Recent studies in the last few years also support the trend. Warli and Dhodia, tribal populations in Dadra and Nagar Haveli have a frequency of 10.1%⁽²⁶⁾ and 13.5%⁽²⁵⁾, respectively, while Rajput, caste group from the same geographical region have low frequency of 2.1%⁽²⁷⁾. Saraswathy and Sachdev have also reported the high frequency of G6PD deficiency among two tribal populations – Koyadoras (8.45%) and Nayakpods (10%) of Andhra Pradesh⁽²²⁾. Saraswathy and Shweta (2005) reported the frequency of G6PD deficiency low among Tamil Brahmin (4.4%)⁽²³⁾. Prevalence of G6PD deficiency in the Indian community was first reported from the Parsi population of Mumbai in the year 1963 by Baxi et al⁽¹⁶⁾. The prevalence rate of G6PD deficiency varies between 0–28% in different caste, tribe and ethnic groups. The highest frequency (27.94%) has been reported from Vataliya Prajapati from Surat, Gujarat^(13,20). The Parsi population of Mumbai also shows high frequency.^(13,20) However, high prevalence of 27.1% reported among Angami Nagas of Nagaland by Seth and Seth⁽¹⁷⁾ has not been replicated in the study of Saha et al.⁽²⁴⁾. The qualitative or semiquantitative methods might be not sufficient to detect all heterozygote females^(12,18). One study from Greece reported that a high percentage of partially deficient female neonates are missed during routine semiquantitative method, which uses a cut off of 2.1 U/gHb. This study proposed a fully quantitative G6PD screening kit and suggested that any neonate with an activity below 6.4 U/gHb should be considered as G6PD deficient⁽¹²⁾. In the present study, the first report of G6PD deficiency among the all neonates born in Ims & sum was made using a fully quantitative method and a cut off of 6.4 U/gHb. In our study, the male to female ratio in G6PD deficient neonates was 3:1

A study conducted on molecular variants of G6PD deficiency among certain tribal communities of Orissa revealed that, 6.4% of males were G6PD deficient⁽¹¹⁾.

CONCLUSION

It is essential to find out the frequency of G6PD deficiency among the different ethnic groups living in Orissa, because The World Health Organization recommends screening all new-borns in populations with a prevalence of 3–5% or more in males⁽¹¹⁾. Routine neonatal screening in Orissa, due to its relatively high prevalence of G6PD deficiency, is logical. Other hand, an increase incidence of the disease complications, such as kernicterus and haemolytic anaemia, would probably occur⁽¹⁹⁾. These changes could include the implementation of new born screening of at-risk population, observation of affected new-borns for neonatal complication, the rapid treatment and more importantly the initiation of counselling for G6PD-deficiency families aiming at increasing awareness of haemolytic triggers.

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