ORIGINAL ARTICLE

GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD)ENZYME DEFICIENCY IN JAUNDICED INFANTS IN KHYBER PAKHTUNKHWA

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ABSTRACT

Objective: To evaluate the frequency of G6PD deficiency by a G6PD quantitative method in jaundiced infants coming from different districts of Khyber Pakhtunkhwa.

Material and Method: This was descriptive cross sectional study done in the departments of Pathology, Hayatabad Medical complex and rehman Medical Institute of Peshawar, from February 2016 to September 2016. 300 (Male n=200, Female n=100) hyperbilirubinemia infants of < 01 year were randomly selected. Through aseptic technique 4 ml blood was collected for bilirubin and G6PD enzyme assay. Serum bilirubin was determined by Diazo reaction method using Architect plus ci8200 (Abbott) and G6PD quantitative enzyme assay was measured by the Ultraviolet kinetic method (Trinity biotech kit, USA). Data were recorded and analyzed on SPSS-20.

Results: In the present study 33 (11%) jaundiced infants were G6PD deficient and 267 (89%) infants had normal G6PD level. In male jaundiced neonates 25 (12.5%) were G6PD deficient and 175 (87.5%) with normal level of G6PD. In female jaundice infants 08 (08%) were G6PD deficient while 92 (92%) had normal G6PD level. No significant differences in the G6PD enzyme level were seen among male and female jaundiced infants. Mean serum total bilirubin in G6PD deficient neonates and normal infants was 13.8 mg/dl and 12.2 mg/dl respectively.

Conclusion: The present study revealed that infants presenting with jaundice have a high percentage G6PD deficiency. As in our region, malaria is endemic and poverty leads to frequent episodes of infection, certain drugs can cause fatal hemolysis.

Key Words: G6PD deficiency, jaundice, infant, Bilirubin, Oxidative drugs

This article may be cited as: Khan S, Mir A, Arif S, Khattak BR, Raziq F. Glucose 6 Phosphate Dehydrogenase (G6PD) enzyme deficiency in jaundiced infants in Khyber Pakhtunkhwa. J Med Sci 2017; 25: (2) 84-87.

INTRODUCTION

Glucose 6 Phosphate Dehydrogenase (G6PD) deficiency is a multi ethnic sex- linked recessive, inherited gene disorder and most frequent red cells enzymopathy of the world[1]. G6PD enzyme protects RBC from destruction in response to oxidative damage[2]. G6PD catalyzes the first and rate limiting step in the pentose phosphate pathway to generate nicotinamide adenine dinucleotide Phosphate (NADPH) that is subsequently utilized in oxidative stress in RBC[3]. G6PD deficiency was first time discovered in 1956 by Alving and his colleagues in the search of hemolytic anemia occurring in some individuals treated with Primaquin (Anti-malarial) in Blacks. Later it was known that G6PD deficiency not only occurred in Africans (Blacks) but was prevalent worldwide. Recently it has been documented that approximately 400 million peoples are G6PD deficient globally[4].

G6PD enzyme activity is controlled by G6PD gene located on the long arm of the Sex linked chromosome [Xq28] neighbor to the gene for factor VIII and color blindness[4]. The G6PD gene is X-lined recessive in its pattern of inheritance and consists of 13 exons and 12 interons that encode a 515 amino acids monomer. Active G6PD enzyme exists in homo-dimmers or tetramers.[5] Males are hemizygous and have only one gene copy for G6PD on X chromosome. The G6PD gene in males can be expressed normally or abnormally to make them G6PD diffident. Females contain two copies of G6PD genes on each Sex-chromosomes and so they

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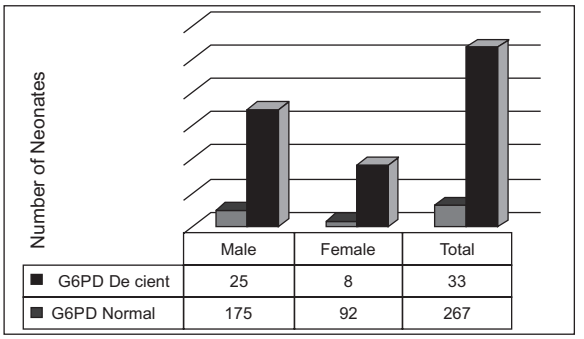
Date Received: October 5, 2016
Date Revised: November 20, 2016
Date Accepted: February 10, 2017



Clinically G6PD deficient individual complain of fatigue, back pain, anemia, jaundice and black urine[9]. Peripheral blood film morphology shows normocytic normochromic red blood cells with fragmented RBCs (typically bite and blister cells), spherocytes, polychromasia and nucleated RBCs may be present, elevated unconjugated bilirubin, lactate dehydrogenase and reticulocyte count. Several screening tests for G6PD are available such as dyecolorization test, monospotfluerocent spot test, methhemoglobine reduction test and farmazon test.[4] and [10] G6PD gene mutation variants and females carrier status can be identified by Polymerase

Table 2: Serum bilirubin comparative analysis in G6PD deficient neonates and G6PD normal neonates in different gender

Study Group					Control Group
G6PD status	Gender	SBR (mg/dl)	Sig	N	Serum Bilirubin
G6PD normal infants	Male	10.01± 5.677	0.118	175 (58.33%)	0.93 ± 0.29
	Female	12.84 ± 3.96	0.873	92 (30.66%)	
G6PD deficient infants	Male	12.68 ± 5.95	0.489	25 (8.33 %)	
		15.50 ± 7.19	0.947	8 (2.66%)	

Table 02: List of medicines shows association with acute hemolysis in G6PD deficiency patients.

	Definite Association	Possible Association	Doubtful Association
Anti Malarial	Primaquine	Chloroquine	Mepacrine
	Pamaquine		Quinine
Antipyretic/ analgesic	Acetanilide	Aspirine	Paracetamol
			Phenacetine
Sulfonamides	Sulfanilamide	Sulfasalazine	Aldesulfone
	Sulfacetamide	Sulfadimidine	Sulfadiazine
	Sulfapyridine	Glibenclamide	Sulfafurazole
	Sulfamethoxazole	…..	……
Sufones	Dapsone	…..	……
Other Drugs	Nalidixic acid	Cifrofloxacine	Aminosalicylic acid
	Niridazole	Chloramphenicol	Aminosalicylic acid
	Methylthionium	Vit. K Analogues	Probenecid
	Phenazoppyridine	Ascorbic Acid	Dimercaprol
	Co- Trimoxazole	Mesalazine	……

can be normal, heterozygous or rarely homozygous. Heterozygous females are the carrier of defective gene has normal, intermediate or very low G6PD enzyme activities, depending on genetic mosaicism occurring as a result of X chromosome inactivation. G6PD deficiency is due to one or more mutations in the G6PD gene leading to functional variants of the proteins resulting in different phenotypes. More than 400 mutations have been reported so far. Nucleotide mutation G6PD A-(202A>G) variant is more frequent in Africans and G6PD 563C>T variant is more common genotype in southern Europe, Middle East and Indian subcontinent.[4,6]

chain reaction- restriction fragment length polymorphism (PCR-RFLP) or Denaturing high performance liquid chromatography (DHPLC).

The aim of this study was to determine the prevalence of G6PD deficiency by G6PD quantitative method in jaundiced infants coming from different districts of Khyber PakhtoonKhwa (KPK).

MATERIAL AND METHODS

Duration of the study was eight months, i.e. from February, 2016 to September 2016. In this descriptive

cross sectional study total no of three hundred (Male n=200, Female n=100) infants were included with presenting neonatal jaundice in the Rehman Medical Institute hospital KPK Peshawar. Only those hyper bilirubinemic infants were enrolled in this of less than 01 year having, hyperbilirubinemia infants were randomly selected for this study. Four ml blood was collected from all infants, 2ml blood in EDTA tube (Purple Top) for G6PD quantitative enzyme assay and 2ml in a non anticoagulated tube (Red Top) to determine serum bilirubin profile. Non anticoagulated tube was centrifuged for 20 minutes and clear serum was separated for measurement of bilirubin estimation. Bilirubin profile was determined by Diazo reaction method (Architect plus ci8200, Abbott, USA). Elevated serum bilirubin infants'samples was further processed for determination of G6PD quantitative enzyme assay. G6PD quantitative enzyme assay was measured by ultraviolet kinetic method (Trinity Biotech G6PD kit, USA). The collected data was recorded and analyzed in SPSS-20. Mean + SD and CV were calculated for numerical variables while frequency and percentages were calculated for categorical variables. All results were presented in the form of tables. P value was less than 0.05, it will consider as statistical significant.

RESULTS

The study was conducted in Rehman Medical Institute Hospital Peshawar (KPK). Total number of jaundiced infants enrolled in this study were 300, among them 200 (66.65%) males and 100 (33.35%) females. The study group ages range between 0 day - 360 days and mean age being two weeks.In this study 33(11%) jaundiced infants were G6PD deficient and 267(89%) were normal G6PD level. In all jaundiced male neonates, 25 (12.5%) were G6PD deficient and 175 (87.5%) infants with G6PD normal level, while 8 (8%) female infants were G6PD deficient and 92 (92%) were G6PD normal in all jaundiced female infants as shown in Table 1.

The G6PD enzyme level in all (n=300) infants range from 0.62 U/g Hb to 25.0 U/g Hb with mean range 12.2U/g Hb. No significant differences in G6PD enzyme level were seen among male infants and female infants. Total serum bilirubin level in this study range from 1.1 mg/dl to 40.4mg/dl with mean range 12.21mg/dl. The mean serum bilirubin in G6PD deficient infants and G6PD normal infants were 14.09 mg/dl and11.6 mg/dl respectively. Study result shows that G6PD level has no statistical significant correlation were seen among G6PD level and serum bilirubin level in studied population, serum bilirubin comparative analysis in G6PD deficient neonates and G6PD normal neonates in different gender as shown Table 2. Insignificant correlation between serum bilirubin and G6PD assay may due to causes of hyperbilirubinemia in neonates other than G6PD enzyme deficiency.

DISCUSSION

This study was conducted to evaluate G6PD enzyme deficiency in neonates by quantitative assay in Pakhtoon Population residing in malaria endemic area of Pakistan. Across the world there are geographic and racial differences in the prevalence of G6PD deficiency and Meta analysis showed that about 4.5% of global populations were G6PD deficient. G6PD enzyme deficiency is more common in Africans , Asia, Mediterranean countries and Latin America due to Prevalence of the deficiency and it correlation with the geographic distribution of malaria[4].

In Asian population Northern Lao-Thai border has high (20%) prevalence of G6PD deficiency while Egypt has low (01%) prevalence for G6PD deficiency. The distribution of G6PD deficiency among the Asian countries varied widely to each other, G6PD deficiency data analysis showed 2-3% for Myanmar and Philippines, 2-5% for Indians subcontinent, 5-9% for Thailand, 10.2% for Azerbaijan Jan and 11.5% for Iran.[11, 12]

Many G6PD enzyme deficient individuals are unaware their G6PD status and asymptomatic in their whole life. G6PD defiant individuals lead to onset of acute hemolysis, when red blood cells exposed to oxidative stress due to some factors such as drugs (Primaquin), foods (Favabean, Pumpkin) and infections (Hepatitis A & B, Cytomegaly virus, Pneumonia and typhoid). Some clinical disorders have been reported which can cause hemolysis in G6PD deficient individuals such as diabetes, Myocardial infarction and strenuous physical exercise, but main underlying cause of acute hemolysis is oxidant drug exposure or coexistent infections[6,13 and 14].

Several medicines interlinked to acute hemolysis in G6PD deficient individuals but it is difficult to specify medicines responsible for hemolytic crisis in G6PD deficient individuals due to some reasons as association shown in table 02. Firstly some medicines safe for some G6PD deficient individuals but it is not necessarily safe for all G6PD deficient individuals because pharmacokinetics can vary individual to individual. Secondly some medicines administrated to the G6PD deficient patient with clinical conditions (such as infection) can produce potentially oxidant effects leading to hemolytic crisis. Thirdly mostly G6PD deficient patient is taking more than one type of medications. Fourthly acute hemolysis in G6PD deficient patients is self limiting process and most of the times do not correlate with clinically significant anemia and reticulocytosis.[15]

CONCLUSION

G6PD deficiency is directely related to janudice in infant population.

RECOMMENDATIONS

So as per WHO protocol our population needs routine screening for G6PD deficiency. We suggest that quantitative G6PD levels should be done at least prior to anti-malarial therapy in every infant. Therefore, we suggest that quantitative G6PD levels should be performed prior to suggestion of anti-malarial therapy.

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CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE NIL

AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Khan S: Main idea.
Mir A: Manusript writing and data analysis
Arif S: Bibliography, data analysis
Khattak BR: Literature search.
Raziq F: Critical review.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.