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Research Article

DRUG INDUCED HEMOLYTIC ANEMIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS IN MALARIA

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ABSTRACT
Glucose-6-phosphate dehydrogenase deficiency is a common hemolytic genetic disorder, particularly in the areas endemic to malaria. The risk of drug induced Glucose-6-phosphate dehydrogenase deficiency related hemolysis depends on a number of factors including the Glucose-6-phosphate dehydrogenase variant. Aims: To know the G6PD deficiency and identify its variants in drug induced hemolysis in malaria cases. Settings and Design: Over a period of two years, 106 clinically suspected cases of DIHA, from Visakhapatnam district were reported of which 87 cases (the study group) were diagnosed as malaria. Materials and Methods: Eighty seven cases of malaria with suspected drug induced hemolytic anemia were reported. Hemoglobin levels, reticulocyte counts and serum indirect bilirubin levels were estimated. G6PD deficiency was detected and the enzyme activity in erythrocytes was estimated. The mutation underlying G6PD deficiency was identified. Statistical analysis used: Percentages and p-values were calculated. Results: The severity of hemolysis varied, causing mild hemolysis to transfusion-dependent anemia. 31 Glucose-6-phosphate dehydrogenase deficient variants were identified. Glucose-6-phosphate dehydrogenase was severely reduced in Mediterranean variant, moderate in Orissa, Kerala-Kalyan and Namoru variants of the enzyme. Hemolysis was moderate to severe among the individuals with Mediterranean mutation. Hemolysis was moderate among individuals with Namoru mutation and mild with Orissa and Kerala-Kalyan mutations. Conclusions: G6PD deficiency was a common occurrence in our setting. G6PD Mediterranean was the most frequently encountered variant producing severe G6PD deficiency. The frequency correlates with malaria endemicity.

Key Words: *Drug Induced Hemolytic Anemia, G6PD Deficiency, Malaria*

INTRODUCTION
Hemolytic anemia after administration of the anti-malarial drug pamaquine (also known as plasmoquine and plasmochin) was reported as early as 1926 (Cordes, 1926). However, it was not until the 1950s that a series of investigations by United States Army researchers identified Glucose-6-phosphate dehydrogenase (G6PD) deficiency as the cause of hemolysis after administration of the related anti-malarial primaquine (Beutler, 1980). In G6PD deficiency, hemolysis could be triggered after ingestion of certain drugs or even after exposure to oxidant free radicals generated by leukocytes in the course of infection (Aly, 2002). The drugs implicated include anti-malarials, sulfonamides, nitrofurantoins etc (Glader and Lukens, 1998). Drug induced hemolytic anemia (DIHA) caused by the administration of certain drugs, especially the anti-malarial drug like primaquine is manifested by a fall in the hemoglobin level after one to two days. India is endemic for malaria and anti-malarial drugs are routinely prescribed even for cases with fever. Thus, correlating the presence of G6PD deficiency with DIHA is of great importance in the Indian population. However, there are only a few case reports of DIHA following ingestion of drugs in G6PD deficient individuals from India and only the biochemical characterization of the G6PD variants in question was carried out in these studies (Bhattacharya and Mitra, 1984). G6PD is remarkable for its genetic diversity. Many variants of G6PD, mostly produced from mis-sense mutations, have been described with wide ranging levels of enzyme activity and associated clinical symptoms. Thirteen

biochemically characterized variants have been reported from India. The polymorphic mutations underlying G6PD deficiency in India which were studied in small selected population groups are reported to be G6PD Mediterranean, G6PD Orissa and G6PD Kerala-Kalyan (Kaeda *et al.,* 1995). G6PD Namoru is the major polymorphic variant in the tribal populations in southern India (Chalvam *et al.,* 2007). In patients with more severe forms of enzyme deficiency such as G6PD Mediterranean, young cells are severely deficient in G6PD (Piomelli *et al.,* 1968), and as a consequence, hemolysis continues until well after the administration of drug is stopped (Pannacciulli *et al.,* 1965 and George *et al.,* 1967). Only few studies correlating the mutation underlying G6PD deficiency with DIHA have been reported from the Indian population. With this background the present study was conducted with an aim to know the G6PD deficiency and identify its variants in drug induced hemolysis in malaria cases.

MATERIALS AND METHODS

The study was done in Visakhapatnam of Andhra Pradesh state in India which is an endemic region for malaria. One hundred and six clinically suspected cases of DIHA, all in the age group 5 to 42 years from Visakhapatnam district were reported over a period of two years. Out of these, a total of 87 cases (the study group) were diagnosed as malaria and received anti-malarials. The dosage of choloroquine was 10 mg/Kg-body weight, adult dose 600 mg; primaquine was 0.75 mg/Kg body weight, adult dose 45 mg and quinine was 542 mg base (650 mg sulfate salt) orally three times a day for seven days. In all the malaria cases, hemoglobin (Hb) levels, reticulocyte counts (new methylene blue supravital staining method), serum indirect bilirubin levels by substracting serum direct bilirubin from serum total bilirubin (Modified Jendrassik & Grof's method) were estimated. The number of units of blood transfused was recorded at the time of the hemolytic crisis. They were screened for G6PD deficiency. The erythrocyte enzyme levels were estimated after the subjects reached a stable state. G6PD deficiency was detected by Nitro blue tetrazolium (NBT) test (George *et al.,* 1967) and the enzyme activity was estimated by WHO method (WHO scientific group, 1967). Thirty one cases of malaria were found to be G6PD deficient and 56 cases as G6PD non-deficient. The mutation underlying G6PD deficiency was identified by polymerase chain reaction (PCR) and restriction enzyme digestion (Kaeda *et al.,* 1995). DNA extraction and analysis was carried out when the subjects came for follow-up almost three months after the crisis. Mean and standard deviation (SD) were calculated on Excel software of MS Windows. Statistical analysis was done with the help of SPSS (Statistical Package for Social Sciences) version 17.0 to get the p-values.

RESULTS AND DISCUSSION

Eighty seven cases diagnosed as malaria had received drugs like primaquine, chloroquine and quinine. None of the subjects included in this study showed a history of anemia or jaundice following ingestion of fava beans. Table 1 shows hematological parameters of the 87 malaria patients i.e. 31 variants and 56 G6PD non-deficient individuals. The Hb, serum indirect bilirubin, reticulocyte count as well as G6PD values of the variants were of a lower range as compared to G6PD non-deficient individuals.

Table 1: Hematological parameters of the 87 malaria patients (31 variants and 56 G6PD non-deficient individuals with DIHA)

Hematological parameter	G6PD variants (n=31) Mean±SD	G6PD non-deficient (n=56) Mean±SD	Z-value	p-value
Hemoglobin (g/dl)	6.50±2.19	8.46±0.64	4.8693	*<0.0001
Reticulocyte count (%)	5.48±2.69	3.52±0.63	3.9966	*<0.0001
Serum indirect bilirubin (mg/dl)	3.09±1.82	1.15±0.31	5.8878	*<0.0001
G6PD IU/g Hb	1.50±0.71	8.98±2.16	23.7042	*<0.0001

**Highly significant*

The G6PD variants showed lower values of Hb, serum indirect bilirubin, reticulocyte count as well as G6PD as compared to G6PD non-deficient individuals. The difference for all the above three parameters was statistically highly significant (p-value<0.0001). Literature review did not reveal any study comparing G6PD variants with G6PD non-deficient individuals. Decrease in the hemoglobin level is the most readily ascertained sign of drug induced G6PD deficiency related hemolysis and is easy to monitor. The severity of hemolysis depends on the variant type and extent of enzyme deficiency. It was observed that G6PD deficient subjects had a varied degree of hemolysis and showed mild to severe anemia. Reticulocyte count was increased during hemolytic crisis. G6PD enzyme deficiency can cause jaundice at birth or chronic hemolytic anemia in the later life. Indirect hyperbilirubinaemia was also noted in majority of the cases.

Table 2 shows distribution of G6PD variants depending upon the anti-malarial drugs used. G6PD deficiency was detected in 31 patients of whom 22 were characterized as G6PD Mediterranean type, four cases as G6PD Orissa, four as G6PD Kerala-Kalyan and one case as G6PD Namoru.

Table 2: Distribution of G6PD variants with DIHA depending upon the anti-malarials

Drugs used	Number	Percentage
Only Primaquine	17	54.84
Primaquine+Chloroquine	9	29.03
Primaquine+Quinine	3	9.68
Mixed anti-malarials	2	6.45
Total	31	100

In the current study, primaquine administration alone resulted in hemolysis in more than half of the cases. Drugs that cause hemolysis in G6PD deficient persons inflict oxidative damage to erythrocytes leading to erythrocyte destruction. Primaquine was the index drug for another study (Sukumar *et al.,* 2004) in drug induced hemolysis in G6PD deficiency. In patients with more severe forms of enzyme deficiency such as G6PD Mediterranean, young cells are severely deficient in G6PD (Piomelli *et al.,* 1968) and as a consequence, hemolysis continues until well after the administration of drug is stopped (Pannacciulli *et al.,* 1965; George *et al.,* 1967).Table 3 shows mean values of hematological parameters of G6PD variants with drug induced hemolytic anemia. The hemoglobin levels ranged between 3 to 9.4 g/dl, reticulocyte count from 3% to 11% during the hemolytic crisis. Serum indirect bilirubin was noted in the range 0.9 to 6 mg/dl. 24 individuals with variants had received blood transfusion (1-6 units). The results of quantitative assay showed G6PD enzyme activity range as 0.71 to 3.67 IU/g Hb (reference range for G6PD measured at 37°C is 4.6 to 13.5 IU/g Hb).

Table 3: Details of hematological parameters of 31 G6PD variants with DIHA

G6PD variants	Hemoglobin (g/dl) Mean±SD	Reticulocyte count (%) Mean±SD	Serum indirect bilirubin (mg/dl) Mean±SD	G6PD IU/g Hb Mean±SD
G6PDMediterranean (n=22)	5.99±2.09	6.05±2.72	3.56±1.68	1.18±0.29
G6PD Orissa (n=4)	8.62±0.60	3.25±0.64	1.20±0.41	2.48±0.77
G6PD Kerala-Kalyan (n=4)	7.25±2.48	5±2.35	2.33±2.12	2.21±0.88
*G6PD Namoru variant (n=1)	6.2	4	3.3	3.3
All variants (n=31)	6.50±2.19	5.48±2.69	3.09±1.82	1.49±0.71

**Only one case detected*

In the present study, observations indicate that some individuals had very severe crises requiring multiple transfusions, while the others had relatively mild hemolysis, which was self-limiting. The commonest G6PD deficient variant found was the Mediterranean variant. Sukumar *et al.,* (2004) in their study also observed that G6PD Mediterranean had a more widespread distribution as compared to G6PD Kerala-Kalyan and G6PD Orissa and G6PD Mediterranean was found to have significantly lower red cell enzyme activity and more severe clinical manifestations than the other two. It is known that Mediterranean variant of G6PD due to C563 CT point mutation results in acute hemolysis triggered by oxidants (Sukumar *et al.,* 2004; Vulliamy *et al.,* 1988). However according to Vulliamy *et al.,* (1988) in the Middle East and India, the clinical symptoms of G6PD Mediterranean mutation are mild inspite of severe enzyme deficiency. The present study revealed G6PD enzyme values in Mediterranean type as 0.71 to 1.52 IU/g Hb, G6PD in Orissa type was 1.26 to 3.19 IU/g Hb and in Kerala-Kalyan type it was 1.38 to 3.67 IU/g Hb. These findings are consistent with other studies. (Nishank SS *et al.,* 2005) in their study observed that erythrocyte G6PD enzyme activity was severely reduced in the case of G6PD Mediterranean type (0.64-1.1 IU/g Hb) as well as among the uncharacterized samples, but was moderate in G6PD Orissa type (1.2-3.1 IU/g Hb). Their study showed that anemia was moderate among the individuals with G6PD Mediterranean mutation and mild among individuals with G6PD Orissa mutation. The normal reference value for G6PD is 4.6 to 13.5 IU/g Hb. The most common variant i.e. G6PD Mediterranean variant showed mean G6PD of 1.18 (8.74% of high normal value 13.5 IU/g Hb). In the present study majority of G6PD deficient subjects had enzyme activity around 10% of the normal value, thus suggesting WHO class II variant (Yoshida A *et al.,* 1971).

The current study has limitations such as clinical outcome of the patients was not studied so that focus can be given to biochemical parameters only. As it was a cross-sectional study, hemoglobin level only at one particular time was taken into account for the study purpose. Hence only low haemoglobin could be determined. A decrease in the values could not be reached upon.

In conclusion, G6PD deficiency was a common occurrence in our setting. G6PD Mediterranean was the most frequently encountered variant producing severe G6PD deficiency. The frequency correlates with malaria endemicity. As drug induced G6PD deficiency related hemolysis depends on the extent of G6PD deficiency and on its molecular genetic basis, there is a compelling need for introducing measures such as genetic counseling and public health education as part of the overall health and welfare services.

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