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G6PD DEFICIENCY AND HAEMOLYTIC ANAEMIA IN URBAN HETEROGENEOUS POPULATION OF BHOPAL

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ABSTRACT

G6PD deficiency one of the most common enzyme deficiency disorder is associated with hemolysis of RBCs. Deficiency of the enzyme fails to impart protection against oxidative stress due to infection , therapy or dietary factors leading to hemolysis of RBCs. The Present study investigates the incidence of G6PD and correlates it to alterations in the Hemoglobin count and reticulocyte count non tribal, hospital visiting population of Bhopal. In the study patients visiting three hospital of Bhopal were analyzed for G6PD deficiency and standard tests for the detection of the deficiency and other tests like Reticulocyte count and Hemoglobin estimation were done for the purpose of correlation. The study found a significant difference in the incidence of disease between males & female. Moreover G6PD deficiency in patients also showed a significant covering of Hb and a significant increase in the reticulocyte count as compared to normal (control) subjects. The results of the present study were well in agreement with similar studies throughout the world. The study concludes a severe health hazard due to G6PD deficiency not only in tribal rural population but also in heterogeneous urban population of Bhopal which may be due to endogamy and tribal connection such groups.

KEYWORDS: **G6PD, X-linked trait, hemolytic anemia, methemoglobin , reticulocyte**

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INTRODUCTION

Glucose-6-phosphatedehydrogenase(G6PD) deficiency is one of the most prevalent enzyme deficiency in human populations affecting nearly 400 million people worldwide.[1] It has been found that the most common ailment associated with G6PD deficiency is hemolytic anemia, a condition in which rythrocytes are destroyed faster than the body can replace them. The G6PD enzyme catalyzes the first step in the pentose phosphate pathway, leading to production of antioxidants that protect cells against oxidative damage.[2] Therefore, in case of G6PD deficiency, ability to protect erythrocytes against oxidative stresses from certain drugs, infections[26, 27] and ingestion of fava beans is impaired leading to premature lysis of erythrocytes.[3]The deficiency is an X-linked trait or genetic defect caused by mutations in the *G6PD* gene. The inheritance of G6PD deficiency shows a typical X-linked pattern with higher incidence in males than in females. [4] A candidate gene encoding glucose-6-phosphate dehydrogenase (G6PD), is a "housekeeping gene" that cods for the enzyme that participates in the glycolytic pathway and for the maintenance of the balance of NADPH, an important cofactor for cellular detoxification.[24,25] It has been found that Certain ethnic groups have high prevalence of G6PD deficiency than others. Moreover the severity of G6PD deficiency also differs among different populations and has been extremely studied among tribal populations.[12,13] In India, the prevalence of G6PD deficiency varies between 0-27% in different caste, tribe and ethnic groups. Studies have ,shown that prevalence is higher among the tribals in comparison to non-tribal caste populations.[5,6] In Connection to this the Prevalence of G6PD deficiency in urban and hospital attending heterogeneous populations of selective regions in India has also been reported.[7,8,9] The aim of the present study was to assess the G6PD deficiency and its clinical consequences with special reference to hemolytic anemia in urban heterogeneous population of Bhopal, M.P, India. This study was undertaken in background of the fact that G6PD deficiency is predictive of an increased risk for adverse health effects that involves hemolytic anemia.

MATERIAL AND METHODS

All works were conducted under the supervision of qualified medical personnel and with permission and certification from Institutional Ethical Committee for laboratory practice (Reference No. IEC/BU/Q1 dated-09.01.2012). Also consent from the patients/subjects were taken in written for the purpose.

Subjects

Subjects and their families frequently attending hospitals of Bhopal city for remedies for their ailments were identified for the present study. The subjects attending three selective hospitals of Bhopal namely, Carewell Hospital, Chirayu Hospital and Jeevandhara Hospital were sampled for the present investigation.The subjects who suffered from hemolytic anemia as diagnosed by the physicians were also chosen for follow up diagnostic tests. Identification and other essential details of the patient were recorded in structured schedules. Family members of G6PD deficient patients were also tested. A total of 1020 individuals were chosen for the present study. This included 678 males and 342 females. There were four infants, 51 children and 965 adults. All the infants were male whereas among 51 children there were 28 males and 23 females. A total of 965 adults consisted 678 males and 342 females. Ethical clearance for the present study was obtained from Intuitional Ethical committee (IEC) Barkatullah University, Bhopal, India. The Study participants were recruited after providing their voluntary informed consent. Professional health personnel examined the patients.

Collection of blood samples

Blood samples were collected in EDTA vials using disposable syringes and needles from each subject, after obtaining informed/written consent in the presence of a doctor from the selective subjects for G6PD deficiency and other necessary routine hematological tests using standard techniques. Samples of the subjects with G6PD deficiency were used for more elaborate laboratory testing that involved assays related to hemolytic anemia.

Detection of G6PD Deficiency

The qualitative enzymatic activity of G6PD was assessed by DCIP (Dichlorophenol Indophenol) decolorizing test following Bernstein (1962).[10] In this test NADPH evolved through the action of G6PD reduces the dye DCIP into a colorless state ($DCIPH_2$). The rate and the degree of this decolorization are proportional to the G6PD activity in the RBCs. Phenazone methosulphate (PMS) is used as an electron carrier between NADPH and 2,6 DCIP in this test. 20 µl of whole blood followed by 0.5 ml of dye solution were mixed in a test tube containing 1 ml of triple distilled water and immediately overlaid by liquid paraffin, allowed to stand at room temperature. The color of the mixture changes from blue to red in presence of G6PD within 20 minutes for normal while it takes 90-120 minutes for deficient samples. The dye solution was prepared from the stock solutions containing 2 ml G6P anhydrous (50 mM/L), 10 ml NADP+ (3mM/L), 8.7 mg 2,6 DCIP (0.5 mM/L), 60 ml Tris HCl buffer (750 mM/L) and 20 ml distilled water containing 0.4 mg PMS, freshly prepared, forming the whole 92 ml is stored in dark reagent bottle at 4C.

Hemoglobin Estimation

Hemoglobin Estimation was carried out by colorimeter following standard Cyanmethemoglobin method of Dacie and Lewis (1966) [11] which is recommended by International Committee for Standardization in Hematology. The principle of this method is that when blood is mixed with a solution containing potassium ferricyanide and potassium cyanide, the potassium ferricyanide oxidizes iron to form methemoglobin. The potassium cyanide then combines with methemoglobin to form cyanmethemoglobin, which is a stable color pigment read photometrically at a wave length of 540nm. This measures all forms of hemoglobin except sulfhemoglobin and can be easily standardized. Moreover, cyanmethemoglobin reagent also known as Drabkin's solution remains exceedingly stable. Normal hemoglobin level ranges 13.5 to 17.5 g/dl for

men, whereas, this ranges 12.0 to 15.5 g/dl for women. Normal ranges for children vary with age and sex. The

Reticulocyte count

Reticulocyte counting is of great diagnostic and prognostic value in hemolytic anemia's, routinely and widely used in the laboratory' to evaluate bone marrow erythropoietic activity. The manual method of reticulocyte counting was done following Lewis et al (2006) [12] by looking at BCB stained slide under the microscope and counting the number of reticulocytes in a number of fields of view. In this procedure, 100 µl brilliant cresyl blue solution prepared in saline was added to 250 µl of blood, and incubated at 37ºC for 20-25 min. After the incubation with the BCB dye, blood smears on glass slides were prepared for reticulocyte counting. Slides were observed under the microscope (100x). Well defined reticulocytes possess RNA filaments or granules.The normal fraction of reticulocytes in the blood depends on the clinical situation but is usually 0.5% to 2.0% in adults and 2% to 6% in infants.

Data analysis

Data were analyzed using statistical package for social sciences (SPSS) version 20 (SPSS IBM, Chicago, IL). The Chi-squared (X^2) test was used to determine the association between occurrence of hemolytic anemia and G6PD status.

RESULTS

Total of 17 subjects were detected to be G6PD deficient in the present study hence, the overall incidence of G6PD deficiency was 1.67% (Table 1). Comparing the groups on the basis of gender, the incidence of G6PD deficiency was higher in males compared to the females. The incidence noted in male and female subjects were 1.4% and 0.30% respectively. 82.4% G6PD deficient subjects were male as compared to 17.6 female G6PD deficient subjects (Table 2). The difference was statistically significant ($p<0.05$).

Hemoglobin levels

Mean Hemoglobin (gm%) levels in G6PD deficient and normal subjects are depicted in Table 3. G6PD deficient and normal subjects showed Mean Hemoglobin values to be 10.09 ± 3.26 and 11.66 ± 3.21 respectively. G6PD deficient subjects exhibited lower mean values as compared to normal. Further, Mean Hemoglobin values hemoglobin value is decreased in anemia and increased in polycythemia.

in male and female G6PD deficient subjects were 10.56± 3.33 and 7.93 ± 2.10 respectively whereas; in G6PD normal subjects the values noted were 12.31± 3.09 and 10.58± 2.88 respectively. Detailed statistical description is provided in Tables 4 and 5. There were differences in G6PD deficient and normal male and female showing higher values in male as compared to female subjects. Table 6 depicts the range of hemoglobin level (gm%) in G6PD deficient and normal subjects. G6PD deficient subjects have generally shown their hemoglobin to be in the range 6-9. gm% (47.1%) and 9.5-15 gm% (41.2%) whereas; 56.5% G6PD normal subjects have shown their hemoglobin level in the range of 9.5-15 gm%. A considerable proportion of G6PD normal subjects (23.1%) showed their hemoglobin level in the range of 15-20 gm%. G6PD normal subjects with lower range of mean hemoglobin (6-9. gm%) were only 19.2% as compared to 47.1% G6PD deficient subjects. Hemoglobin levels less than 6 and 20 or more gm% were rare in the observations. Table 7 depicts hemoglobin level (gm%) in all male and female subjects sampled for the present study. This revealed that females were generally anemic and nearly 25% had hemoglobin level less than 9.5 gm%. Hemoglobin levels of 60.9 % females were in the range of 9.5<15 gm% whereas; 6.9% females showed their hemoglobinlevel in the range of 15<20 gm%. Further, male subjects have generally shown their hemoglobin to be in the range 9.5<15 gm% (53.3 %) and 15<20 gm% (32.4%) whereas; 16.4% males have shown their hemoglobin level in the range of 6<9.5 gm%. Hemoglobin levels less than 6 and 20 or more gm% were rare in the observations.

Reticulocyte counts

Reticulocyte counts in G6PD deficient and normal subjects are presented in Table 8. Reticulocyte counts in 76.47% G6PD deficient subjects were >2% whereas; 58.42% G6PD normal subjects were detected to have higher counts It has shown statistically significant difference ($p<0.05$). Reticulocyte count <2% was noted in 23.53% and 41.58% G6PD deficient and normal subjects respectively that exhibited higher values in G6PD normal subjects but the difference was not statistically significant

Table 1
Distribution of G6PD deficient and normal subjects

Gender	G6PD normal	G6PD deficient
Male	648 (63.52)	14 (1.37)
Female	355 (34.80)	3 (0.30)
N (%)	1003 (98.33)	17 (1.67)

Figures in parentheses are percentage

Table 2
Incidence of G6PD deficient and normal enzyme levels in male and female subjects

G6PD Enzyme level	Gender*					
	Male			Female		
	Count	Incidence(%)	Row N %	Count	Incidence(%)	Row N %
Deficient	14	1.4	82.4	82.4	0.3	17.6
Normal	627	61.5	62.5	376	36.9	37.5

Statistically significant difference (p<0.05).

Table 3
Mean Hemoglobin (gm%) in G6PD deficient and normal male and female subjects

Gender	G6PD deficient		Normal subjects	
	Mean Hemoglobin	Std .Deviation	Mean Hemoglobin	Std. Deviation
Male	10.56* (14)	3.33	12.31* (627)	3.09
Female	7.93* (3)	2.10	10.58* (376)	2.88
All (Total)	10.09 (17)	3.26	11.66 (1003)	3.21

Figures in parentheses are sample size*Statistically significant difference (p<0.05).

Table 4
Statistical description of Hemoglobin level (gm%) in G6PD deficient subjects

G6PD enzyme level^	Statistic	Std. Error
Deficient		
Mean	10.0941	.78961
95% Confidence Interval for Mean Lower Bound	8.4202	
Upper Bound	11.7680	
5% Trimmed Mean	10.0212	
Median	9.4000	
Variance	10.599	
Std. Deviation	3.26566	
Minimum	5.50	
Maximum	16.00	
Range	10.50	
Interquartile Range	5.45	
Skewness	.411	.550
Kurtosis	-.862	1.063

^Category based on G6PD qualitative DCIP decolourization test.

Table 5
Statistical description of Hemoglobin level (gm%) in G6PD normal subjects

G6PD enzyme level^	Statistic	Std. Error
Normal		
Mean	11.6581	.09868
95% Confidence Interval for Mean Lower Bound	11.4644	
Upper Bound	11.8517	
5% Trimmed Mean	11.6266	
Median	11.2000	
Variance	9.766	
Std. Deviation	3.21506	
Minimum	5.70	
Maximum	24.30	
Range	20.60	
Interquartile Range	4.80	
Skewness	.769	.077
Kurtosis	4.380	.154

^Category based on G6PD qualitative DCIP decolourization test.

Table 6
Hemoglobin level (gm%) in G6PD deficient and normal subjects

Hemoglobin (gm%)	G6PD enzyme level^			
Range	Deficient		Normal	
	Count (N)	Column N %	Count (N)	Column N %
Less than 6	0	0.0	1	0.1
6<9.5	8	47.1	193	19.2
9.5<15	7	41.2	567	56.5
15<20	2	11.8	232	23.1
20 or more	0	0.0	10	1.0
Total	17	100	1003	100

^Category based on G6PD qualitative DCIP decolourization test.

Table 7
Hemoglobin level (gm%) in male and female subjects

Hemoglobin (gm%)	Gender			
Range	Male		Female	
	Count (N)	Column N %	Count (N)	Column N %
Less than 6	0	0.0	1	0.3
6<9.5	105	16.4	96	25.3
9.5<15	342	53.3	231	60.9
15<20	208	32.4	26	6.9
20 or more	6	0.9	4	1.1
Total	662	100.0	358	100.0

Table 8
Reticulocyte count in G6PD deficient and normal subjects

Reticulocyte count	G6PD deficient		G6PD Normal	
	n	%	n	%
>2%	13(17)	76.47*	586 (1003)	58.42*
≤2%	4(17)	23.53	417 (1003)	41.58

Figures in parenthesis indicate sample size*Statistically significant difference (p<0.05).

DISCUSSION

Studies show that prevalence of G6PD deficiency varies according to ethnic variation from 0 (or rare) - 35% in different pockets of human populations in the world. [13,14] The estimate of prevalence, made from data published in 1985 was 7.5% for carriers of mutant alleles and 3.4% were hypothesized to express the deficient phenotype. [13] The 3.4% was based on including all hemizygous male, all female homozygotes and 10% of female heterozygotes. In general, G6PD deficiency has been more frequent in tribes than in non-tribes as evident in many states of India.[15,16,17] This has been found to exist in high frequencies in adult population. Up to 6.7% of neonatal population has also been demonstrated to have this deficiency with or without apparent icterus.[18,19] Considerable variation in the frequencies of G6PD deficiency has been noted in central India. [20] It was 5.88% in Bhilalas of Jhabua[20] while in the same tribal group of West Nimar, it was 10.2%. [17] Frequency of G6PD deficiency in Kawar of Raipur was reported to be 7.83% while it was 16.03%, strikingly higher in the same tribal group of Sarguja. On the other hand, two different endogamous tribes Oraon and Gond of Sarguja district were reported to have invariably high frequencies, 13.38% and 13.02% respectively. The frequency was slightly higher, 15.08% in Gonds of Raipur.[20] Barela tribe in West Nimar was reported to have 9.59% frequency of G6PD deficiency while it was 13.81% in Korku tribe of malaria endemic Pachmarhi hills in Hoshangabad district.[17] A pilot study was carried out to find out the incidence of G6PD deficiency in a randomly selected hospital attending population of Delhi.[21] A total of 300 subjects from OPD of Dr. Ram Manohar Lohia Hospital, New Delhi, were randomly selected and screened for G6PD deficiency. This investigation demonstrated incidence of G6PD to be 3.66% in urban settings whereas; another study on hospital attending population of Delhi demonstrated 1.37% G6PD deficiency,[22] The present study met more or less similar terms and demonstrated 1.67% G6PD deficiency in hospital attending heterogeneous population of Bhopal. The hemolytic manifestation of some G6PD variants can be easily correlated with the extremely low activity of the variant enzymes. The clinical manifestation can sometimes be explained by the unusually low affinity of the variant enzyme for its substrate or coenzyme.[23] Present study is predictive of existence of specific G6PD variants with hemolytic manifestation in diverse ethnic groups of urban Bhopal area in India. Our findings correlate with studies conducted by workers throughout the globe,[4,6] that G6PD deficient patients are anemic having significantly low hemoglobin counts, Moreover our study show that patients with G6PD deficiency have higher reticulocyte count due to the enhancement of erythropoisis owing to the incidence of hemolysis of RBCs. This releases the Immature precursors of RBCs in circulation with the studies by R.D.Bernstein & A. Yoshida.[10,23]

CONCLUSION

Diverse endogamous ethnic groups occupied in heterogeneous population of Bhopal possess G6PD deficiency mutation although, in a low frequency. Population screening has shown that G6PD deficient subjects exhibit lower mean hemoglobin values as compared to normal. Moreover, reticulocyte counts in 76.47% G6PD deficient subjects were >2%. This demonstrates an increased risk for adverse health effects of G6PD deficiency causing hemolytic anemia.

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CONFLICT OF INTEREST

Conflict of interest.

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