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Prevalence of Glucose 6 Phosphate Dehydrogenase Deficiency in Population of Gujarat - Report of 9184 Cases

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Abstract **Case Report**

Background: All humans have the Glucose 6 phosphate dehydrogenase gene. Some people are born with a mutation of the Glucose 6 phosphate dehydrogenase gene. Most of these individuals are asymptomatic but may exhibit non-immune hemolytic anemia, even severe anemia in response to exposure to certain environmental triggers, most commonly, infection or exposure to certain foods like fava beans (favism), medications or chemicals. G6PD deficiency is an X-linked disorder that primarily affects males. Heterozygous females do not usually develop severe hemolytic anemia due to G6PD deficiency. This study destined to reveal the prevalence of G6PD deficiency in south Gujarat population. ***Methods****:* This is a retrospective case study designed to assess the prevalence of G6PD deficiency in gujarat population, for patient requesting G6PD test at multiple collection center of Desai metropolis laboratory pvt ltd. between January 2022 to May 2023. Glucose-6-phosphate dehydrogenase deficiency analysis was done by Methylene dye blue test (Arkray MBK) – Qualitative method. All G6PD deficient patient confirmed by G6PD-quantitative (Kinetic method). ***Results:*** Total 9180 patients (5790 male and 3394 female) were included in this study. They were subsequently categorized into various subgroups and analysed properly. The incidence of G6PD deficiency in the selected sample frame of cases was 3.69 %. In which 4.11% of G6PD deficient cases belong to the male while the rest 2.98 % belong to the female. ***Conclusion:*** Therefore, to conclude whenever clinical and hematological findings raise the suspicion of glucose 6 phosphate dehydrogenase deficiency, the disorder should be confirmed by quantitative/quantitative measurement of red blood cell enzyme activity in both male and female. There is also need for a large screening programme, especially in malaria endemic zones.

Keywords: Glucose 6 phosphate dehydrogenase (G6PD), medications or chemicals.

INTRODUCTION

G6PD is an enzyme present in the cytoplasm of all cells, acting specifically in the maintenance of the integrity of the erythrocytes, preventing the oxidation of hemoglobin and other cellular proteins. Glucose 6-phosphate dehydrogenase (G6PD) deficiency was discovered half a century ago and is still the most common inherited enzymopathy. Clinically, deficiency of this enzyme affects as many as 400 million individuals worldwide [1]. All humans have the Glucose 6 phosphate dehydrogenase gene. Some people are born with a mutation of the Glucose 6 phosphate dehydrogenase gene. Most of these individuals are asymptomatic but may exhibit non-immune hemolytic anemia, even severe anemia in response to exposure to certain environmental triggers, most commonly, infection or exposure to certain foods like fava beans (favism), medications or chemicals [2]. This inherited deficiency causes neonatal hyperbilirubinemia and chronic hemolytic anemia. Depending on the prevalence of G6PD deficiency in a population, neonatal screening programs for G6PD deficiency should be established, aiming for early recognition and prevention of its complications [3]. G6PD deficiency is an X-linked disorder that primarily affects males. Heterozygous females do not usually develop severe hemolytic anemia due to G6PD deficiency [4]. In 1986, the G6PD gene was cloned independently by Persico et al. [5] and Takizawa *et al.* [6]. G6PD gene is located on the long arm of the X chromosome (Xq28), and consists of 13 exons [7]. G6PD locus is thought to be one of the most polymorphic loci among humans with almost 300 allelic variants reported

[8]. The G6PD enzyme monomer consists of 515 residues with over 59 kDa molecular weight. It was reported that the enzymatically active form of G6PD is either a dimer or tetramer of a single polypeptide subunit according to cellular pH [9]. This study was specifically aimed at determining the prevalence of G6PD deficiency in population of Gujarat as evident from a study of 9184 cases.

Detection of G6PD deficiency

Glucose-6-phosphate dehydrogenase (G6PD) enzyme plays a vital role in the generation of NADPH and ribose-5-phosphate in the pentose phosphate pathway [10]. NADPH functions as an electron donor and provides the reducing energy required for regeneration of reduced Glutathione which ultimately protects the cells against oxidative damage. Though G6PD deficiency affects every cell in the body, its primary effects are hematological because in the erythrocyte this pathway is the only source of NADPH [11]. There are various methods available for detection of G6PD deficiency, but quantitative measurement of G6PD enzyme by measuring the reduction of NADP to NADPH using ultraviolet spectrophotometer is the basic diagnostic approach used commonly for detection of G6PD deficiency. Other than that, in a dye reduction test, the reduction of NADPH was linked to the reduction of the visible dye brilliant cresyl blue [10]. Various other tests such as methylene blue, MTT tetra sodium, dichloro-indophenol or methemoglobin were also developed [12, 13]. In recent times, fluorescent spot test is rapid testing of deficiency in which reduction of NADPH is observed directly by virtue of its fluorescence, instead of linking the reduced pyridine nucleotide to a dye [14]. Screening tests involve either quantitative or qualitative measurements of enzyme activity but are affected by several factors [15] and have reduced sensitivity in detecting heterozygous females [16]. Molecular methods provide more consistent results but are costly.

Occurrence of G6PD Deficiency

G6PD deficiency affects around 10% of the total population of world. The highest prevalence of G6PD deficiency mainly regards in tropical Africa, the Middle East, tropical and subtropical Asia, Papua New Guinea and various Mediterranean regions [10, 17, 18]. India, a south Asian country having largest population in the world has varying castes, ethnic and linguistic groups. Also geographically and environmentally India has a great variation, which is also responsible for difference among population. In India, investigations on G6PD deficiency have been started after it was first reported by Baxi *et al.* in 1961 [19]. Since then, various studies regarding prevalence of G6PD deficiency among various population groups have been conducted across India. India being a malarial endemic country, the treatment course requires primaquine drugs which is generally conducted without routine G6PD screening. This makes patients vulnerable to prescription of potentially haemolytic drugs, especially putting G6PD deficient individuals at risk of serious complications. In western India, the investigations of this deficiency have been conducted amongst several tribal as well urban populations comprising of Parsees, Cutchee Bhanushalis, Marathas, Dhangars, Bhils, Pawars, Muslims, Hindus, Jains, Christians, Brahmins, Katkaris, Sikhs, Kayasthas, Bhandaris, Baniyas, Garasiyas, Kumhars, Damors, Minas, Patidars, Rajputs, Bhois, Panchals, Patels, Lohanas, Bohras, Sompuras, Darbars, Harijans, Luhars, Warlis, Dodhias, and many more [20]. Although various investigations have reported occurrence of G6PD deficiency frequenting from 0% to 18% in general, frequency of G6PD deficiency have been reported up to 27.9% in Vataliya Prajapati community from Gujarat which is the second highest in country [21].

MATERIALS AND METHODS

Study design

This is a retrospective case study designed to assess the prevalence of G6PD deficiency in Gujarat population, for patient requesting G6PD test at multiple collection centers of Metropolis Healthcare laboratories Pvt. Ltd. between January 2022 to May 2023. No medical criteria were used to select the subjects.

Sample collection and methods

Blood samples were collected from patients requesting G6PD test belonging to both sexes. Two-milliliters blood was collected from each subject in ethylenediaminetetraacetic acid (EDTA)-anticoagulated vacutainer tubes (EDTA). Glucose-6-phosphate dehydrogenase deficiency analysis was done by Methylene blue dye decolorization test (Arkray MBK) – Qualitative method. All G6PD deficient patients were confirmed by G6PD-quantitative test (Kinetic method).

Principle of by Methylene blue dye decolorization test [22]

Glucose -6 Phosphate Dehydrogenase, present in the red cell hemolysate, acts on Glucose -6 Phosphate and reduces NADP to NADPH which, with the help of PMS, reduces blue colored 2,6 Dichlorophenol indophenol into a colorless form. The rate of decolorization is directly proportional to the enzyme activity (Rate of Decolorization $\propto$ Activity of G-6 PD).

Procedure

Step 1. Preparation of red cell hemolysate: a. Purified water: 2.5 ml, b. Fresh blood: 0.05ml. Mix well and allow standing for 5 minutes at room temperature.

Step 2. Assay of the enzyme:

a. Add 1 ml of the hemolysate (step 1) to the vial of Solution I (Substrate and Buffer with pH 8.5) and mix gently.
b. Add immediately about 2ml of Reagent3 (Mineral oil).

c. Seal the vial with aluminum foil and incubate at 37°C.

Observe: The time taken for the color change from initial deep blue to reddish purple. Follow up to a maximum of 6 hours with 30 minute intervals. *Interpretatio*n: (Decolorization time). Normal Subject regarded as Non Deficient: 30-60 minutes. G-6PD deficient subjects regarded as Deficient (Heterozygous males, homozygous females): 140 minutes to 24 hours. Statistical analysis Statistical analyses were conducted using SPSS (version 11) software. Descriptive analyses of percentages of categorical variables were reported. A P value of <0.05 denotes a statistically significant difference in all statistical comparisons. Correlation was compared using a version of linear regression analysis.

OBSERVATION & RESULT

Table 1: Age and Gender distribution of total no. of cases

Age Group			Gender			
	Total		Female		Male	
	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
<28 days	3696	40.24%	1290	34.90%	2406	65.10%
28 days - 1 Year	131	1.43%	30	22.90%	101	77.10%
1 Year - 15 Year	873	9.11%	331	37.92%	506	57.96%
16 Year - 30 Year	1503	16.37%	538	35.80%	965	64.20%
31 Year - 45 Year	1328	14.46%	518	39.01%	810	60.99%
46 Year - 60 Year	966	10.52%	426	44.10%	540	55.90%
>60 Year	723	7.87%	261	36.10%	462	63.90%
Total	9184	100%	3394	36.96%	5790	63.04%

Table 2: Prevalence of G6PD Deficiency in study population

G6PD	No. of cases	Percentage
Deficient	339	3.69%
Not Deficient	8845	96.31%
Total	9184	100.00%

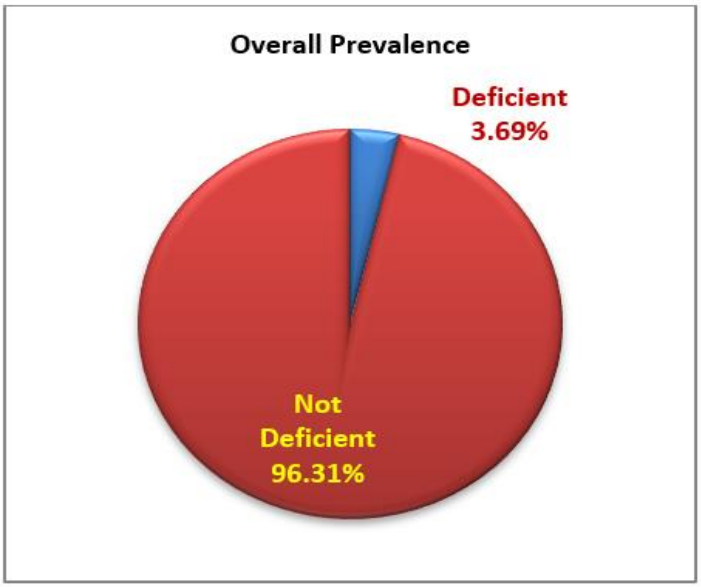

Figure 1: Overall Prevalence

Table 3: Shows the distribution of G6PD deficiency in gender

	G6PD				p value
	Deficient		Not Deficient		
	No. of cases	Percentage	No. of cases	Percentage	
Female	101	2.98%	3293	97.02%	**0.0054**
Male	238	4.11%	5552	95.89%	
Total	339	3.69%	8845	96.31%	

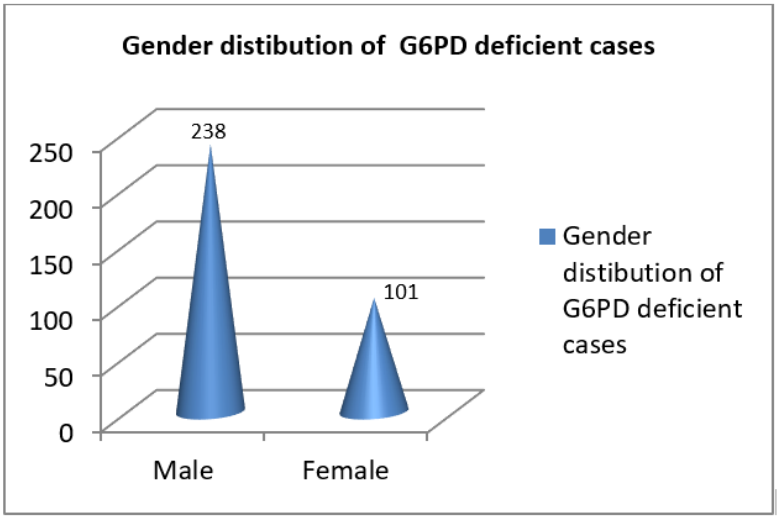

Figure 2: Gender distibution of G6PD deficient cases

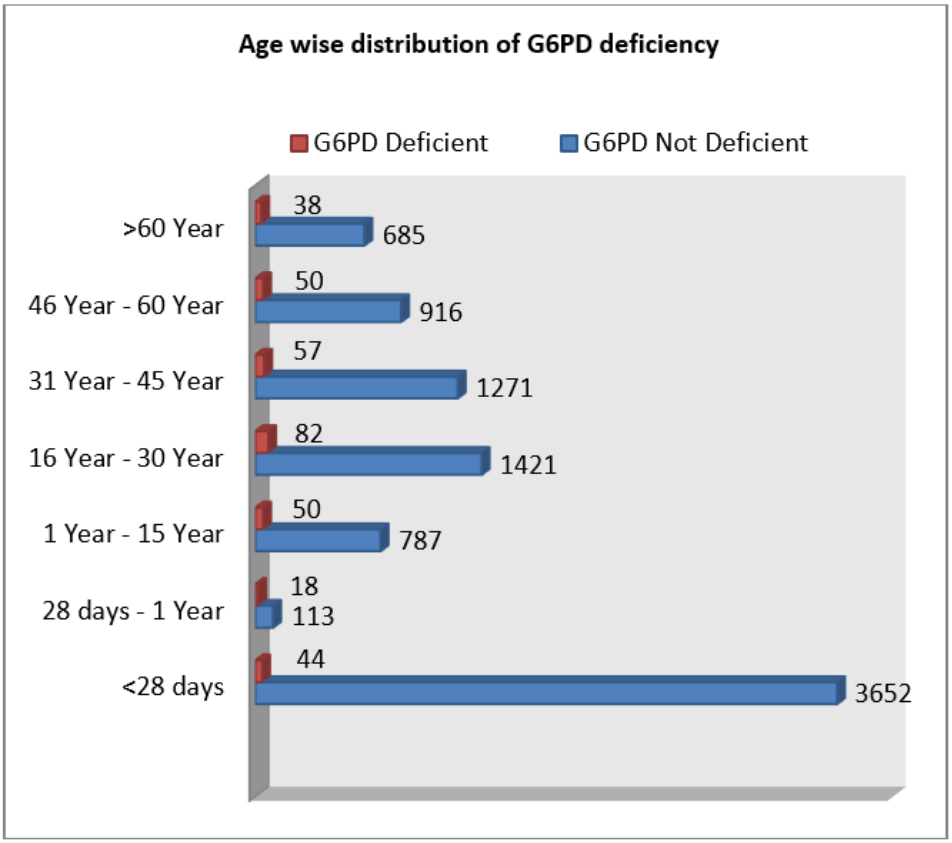

Figure 2: Age wise distribution of G6PD deficiency

Table 1 shows that out of total no. of cases studied (n = 9184), the maximum number of patient was observed in <28 days of age group (40.24%). The age of cases studied varied from new born to 78 years. Out of total no. of cases studied (n = 9184), 63.04 % were males while 36.96 % were females. Table 2 shows Out of total 9184 cases studied, 339 cases showed G6PD deficiency (3.69%). Thus it can be said that prevalence of G6PD deficiency in the selected sample group was 3.69 %, details of which have been shown. As Seen in Table 3, Out of total 9184 cases, 5790 were male subjects. Number of G6PD deficient males amongst them was 238, so prevalence of G6PD deficiency among males studied in our study was 4.11%. Also, out of total 9184 cases, 3394 were female subjects and G6PD deficiency amongst those was present in 101 cases. That means prevalence of G6PD deficiency among females studied in our study was 2.97%. Overall gender wise prevalence of G6PD deficiency in our study was 70.20% in males and 29.79% in females. Out of total 339 deficient cases, we could estimate Hb concentration of 173 cases and noticed that all of them had low Hb concentration.

DISCUSSION

Bhasin and Walter reviewed the prevalence and distribution of Glucose 6 phosphate dehydrogenase deficiency in India by pooling data from 224 different studies based on geographical, occupational, ethnic and linguistic categories. Higher prevalence was reported from North and West than South India [23]. The age of subjects range from new born to 1 year with maximum number of subjects in the age group of 28 days–1 year have highest prevalence in our study. Similar age distribution was also noted in some of the hospital based studies [24, 25]. In our study, 63.04% were males and while 36.96% were females. This study shows that overall prevalence of Glucose 6 phosphate dehydrogenase deficiency is 3.69 %. Out of 3.69% deficient cases, 70 .20% were males and 29.79 % were females. This is consistent with several studies conducted for glucose 6 phosphate dehydrogenase enzyme deficiencies. It is due to the X-linked nature of this genetic disorder. Heterozygous males manifest the disorder while females who are homozygous usually manifest the disorder and heterozygous females remain carriers However, the gender prevalence of G6PD deficiency in our study does not reflect true gender prevalence as our study did not include equal number of male and female subjects. Fortunately, most of the G6PD deficient cases will remain clinically asymptomatic throughout their lives. However, a proportion of glucose 6 phosphate dehydrogenase deficient individuals develop neonatal jaundice or acute hemolytic anemia, which, if managed inadequately, can cause death or permanent neurological damage. The highest frequencies of glucose 6 phosphate dehydrogenase deficiency are in tropical Africa and tropical and subtropical Asia, which are also malaria-endemic areas. In areas of high incidence, clinicians and patients must be alert and prepared to avoid any factors that might trigger severe clinical manifestations of the deficiency.

CONCLUSION

Therefore to conclude When clinical and hematological findings raise the suspicion of glucose 6 phosphate dehydrogenase deficiency, the disorder should be confirmed by quantitative/quantitative measurement of red blood cell enzyme activity. There is also need for a large screening programme, especially in malaria endemic zones, where due to natural selection of population, there seems to be a higher incidence of glucose 6 phosphate dehydrogenase enzyme deficiency, which gives protection against severe malaria.

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