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Prevalence of G6PD deficiency amongst healthy blood donors

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Abstract
Introduction: Blood donation is very common procedure in clinical practice. Deficiency of G6PD is the most common erythrocyte enzymopathy and has been estimated to affect over 400 million people worldwide that may lead to neonatal jaundice or hemolytic crisis due to drugs or infections. By detecting such problem of deficiency of G6PD enzyme, we can prevent complications of blood transfusion. We can decide in whom to avoid such transfusions.
Objectives: To study G6PD deficiency prevalence in healthy blood donors; according to age, sex, ABO blood group and Rh blood Group distribution.
Material and methods: Qualitative method by Methaemoglobin Reduction test (MRT) was used for screening of the blood donors for G6PD deficiency. Quantitative test of all positive donors by qualitative method was done by semi-automated G6PD reagent kit. Observational Cross-sectional study involving 1012 healthy blood donors for period of 9 months was done.
Results: It was observed that 35 (3.5%) blood donors were G6PD deficient amongst 1012 blood donors. The prevalence rate for G6PD deficiency observed in this study was 3.5%.
Conclusion: The observation of 3.5% prevalence of G6PD deficient blood donors in the present study could be taken to consider that the problem of G6PD deficiency exists in the blood donors. It should be of concern as the G6PD deficiency remains obscure, there being no overt clinical manifestation. Prevalence of 3.5% should be taken as serious concern and further more studies are advised for screening of G6PD in healthy blood donors.

Keywords: MeSH terms, G6PD, G6PD deficiency, blood donation, blood donors, methaemoglobin reduction test

Introduction
Blood donation is very common procedure in clinical practice. Due to advancement in technology and precision in testing techniques, we are able to do safe blood transfusions now. But unfortunately sometimes we come across complications of blood transfusion.
Deficiency of G6PD is the most common erythrocyte enzymopathy and has been estimated to affect over 400 million people worldwide that may lead to neonatal jaundice or hemolytic crisis due to drugs or infections [1]. Although global in its distribution, G6PD deficiency is encountered with greatest frequency in the tropical and subtropical zones of the Eastern Hemisphere [2]. G6PD deficiency is the most common enzymatic abnormality worldwide, but its importance in transfusion medicine is relatively understudied and under-appreciated. Blood donors are not routinely screened for G6PD deficiency, which may lead to adverse consequences if G6PD-deficient RBCs are transfused into a susceptible host (e.g. during infection or treatment with an oxidative drug).
Purpose of our study was to screen such donors for deficiency of Glucose 6-phosphate Dehydrogenase (G6PD) enzyme deficiency and its relations with various factors.
Blood bank plays an important role in ensuring the supply of safe blood as and when required to the patients. While it is important to ensure that there is an adequate supply of blood, it is also essential that the collection process does not harm either blood donors or recipients. Blood donation from G6PD deficient donors might alter the quality of the donated blood during processing, storage or in the recipient circulatory system.

So the pre and post donation screening of blood for transfusion transmissible infections and other investigations is mandatory.

According to WHO, individuals with G6PD deficiency with history of hemolysis should defer permanently. While individuals with G6PD deficiency or other inherited red cell membrane defects, without a history of hemolysis are acceptable; however, their blood is not suitable for intrauterine transfusion, neonatal exchange transfusion or for patients with G6PD deficiency [3].

With the changing recommendations for transfusion and treatment regimens for specific patient populations, the relevance of G6PD deficiency in blood donors is increasing, and warrants renewed attention. Determining the prevalence of G6PD deficiency is an essential first step towards evaluating its impact on the health of a population. Early detection and prevention is the key strategy to successful management and control of G6PD deficiency. Genetic counseling, prenatal diagnosis, health education and public awareness can provide benefits by way of preventive genetics to the affected individuals and their families.

By detecting such problem of deficiency of G6PD enzyme, we can prevent complications. We can decide in whom to avoid such transfusions.

Aims and objectives

Aim: To study the prevalence of Glucose-6-phosphate Dehydrogenase deficiency in healthy blood donors.

Objective: To study G6PD deficiency prevalence in healthy blood donors; according to age, sex, ABO blood group and Rh blood Group distribution.

Material and Methods

Method of study – Observational Cross-sectional study

Method of sampling – Randomized sampling

Sample size – 1012 healthy blood donors

Period of study – 9 months (March 2019 to November 2019)

Statistical analysis – The data was done by descriptive statistics for the percentage of G6PD deficiency by age, sex and blood groups.

Place of study – Tertiary hospital and Government Medical College set up. Data regarding donor`s detail was collected from the Department of IHBT.The process of data collection did not cause any potential risk or harm to the participants. Privacy was ensured while collecting data. Only the identified data would be analyzed and presented for the study purpose.

- **Inclusion Criteria:** All samples of Healthy Blood Donors of Blood bank.
- **Exclusion Criteria**
  1. Criteria for rejection of sample: Inadequate sample, Clotted sample, Haemolysed sample.
  2. Donor not willing to participate in the study

Method of study

Qualitative method by Methaemoglobin Reduction test (MRT) was used for screening of the blood donors for G6PD deficiency. Quantitative test of all positive donors by qualitative method was done by semi-automated G6PD reagent kit.

Method

Reagent preparation: Dissolve 5 g of dextrose and 1.25 g of $NaNO_2$ in 100 ml of water. Dissolve 150 mg of methylthionine chloride (methylene blue chloride, sigma) in 1 liter of water. Add 2 ml of anti coagulated blood (EDTA) to the tube containing 0.2 ml of the combined reagents either freshly prepared or dried. Close the tube with a stopper and gently mix the contents by inverting it 15 times.

Prepare control tubes by adding 2 ml of blood to similar tube without reagents (normal reference tube) and to a tube containing 0.1 ml of sodium nitrite-dextrose mixture without methylene blue (‘deficient’ reference tube).

Incubate the samples at 37^0 C for 90 min. After incubation, pipette 0.1 ml volume from the test sample, the normal reference tube and deficient reference tube into 10 ml of water in separate, clear glass test tubes of identical diameter. Mix the contents gently. Compare the colors in the different tubes. Normal blood yields a colour similar to that in the normal reference tube (clear red). Blood from deficient subjects give a brown colour similar to that in deficient reference tube.

Principle of Quantitative method

The enzyme G6PD present in the red blood cells is extracted by lysing the cells using a natural detergent. The extracted enzyme oxidizes Glucose 6 phosphate to 6-phosphogluconate and simultaneously reduces co-enzyme NADP to NADPH giving increase in absorbance at 340 nm.

Procedure

- Determine the hemoglobin content of the whole blood.
- Take 1 ml lysing reagent and 10 ul whole blood in clean test tube. Mix well allow 10 minutes at room temperature. This is hemolysate for assay.
- Take 0.5 ml substrate reagent, 0.5 ml buffer reagent and 0.5 ml above prepared hemolysate into clean test tube. Mix and aspirate immediately and read first absorbance of test exactly at 60 seconds and then second, third and fourth at an interval of 60 seconds at 340 nm. Determine factor for the test and calculate the G6PD activity.
- Less than 6.4 U/g Hb at 37^0 C is called G6PD deficient.

Results and analysis

In the present study, we have tested 1012 blood donor`s samples from blood bank at tertiary medical college set up, for G6PD deficiency; out of which 1005 were male donors and 07 were female donors. It was observed that 35 (3.5%) blood donors were G6PD deficient amongst 1012 blood donors. The prevalence rate for G6PD deficiency observed in this study was 3.5%. Table 1 Shows the status of G6PD according to gender distribution. All 35 G6PD deficient blood donors were male blood donors. All the female blood donors had normal G6PD status.

Table 1: Status of G6PD according to gender distribution

Gender	Status of G6PD				Total	
	G6PD deficiency		Normal G6PD		No	%
	No.	%	No.	%		
Male	35	100	970	99.3	1005	99.3
Female	00	00	07	0.7	07	0.7
Total	35	100	977	100	1012	100

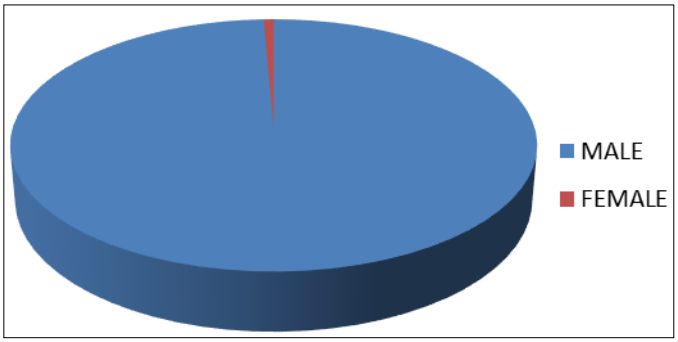

Fig 1: Gender distribution

Table 2 shows status of G6PD according to age group. Out of total 35 G6PD deficient blood donors, maximum 12 (34.3%) blood donors belonged to age group 21-30 years. 445 out of 1012 blood donors belonged to the same group.

Table 2: Status of G6PD according to age group

Age group	Status of G6PD				Total	
	G6PD deficiency		Normal G6PD		No	%
	No.	%	No.	%		
18-20	4	11.4	64	6.5	68	6.7
21-30	12	34.3	433	44.3	445	44
31-40	10	28.6	331	33.9	341	33.7
41-50	6	17.1	121	12.4	127	12.5
51-60	3	8.6	24	2.5	27	2.7
>60	0	0	4	0.4	4	0.4
Total	35	100	977	100	1012	100

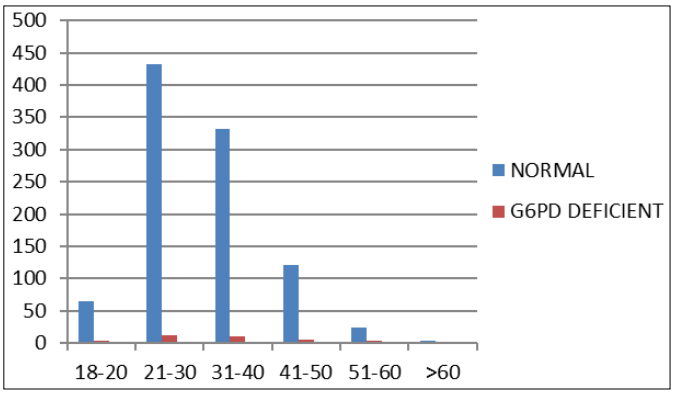

Fig 2: Age wise distribution of g6pd

Table 3 shows the donors' ABO blood group distribution. The most common blood group in the study population was B blood group in both G6PD deficient (15, 42.85%) and G6PD normal (337, 34.5%) blood donors.

Table 3: Donors' ABO blood group distribution and their G6PD status

ABO Blood Group	Status of G6PD				Total	
	G6PD deficiency		Normal G6PD		No	%
	No.	%	No.	%		
A	10	28.6	238	24.4	248	24.5
B	15	42.8	337	34.5	352	34.8
O	7	20	319	32.6	326	32.2
AB	3	8.6	83	8.5	86	8.5
Total	35	100	977	100	1012	100

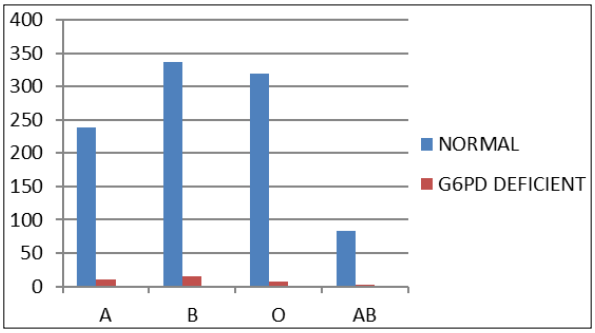

Fig 3: Donors' ABO blood group distribution and their G6PD status

Table 4 shows the Donors' Rhesus blood group distribution and their G6PD status. Out of total 1012 blood donors, 963 blood donors were Rh positive and 49 blood donors were Rh negative. All 35 G6PD deficient blood donors were Rh positive.

Table 4: Status of G6PD according to Rhesus D blood group

Blood group	Status of G6PD				Total	
	G6PD deficiency		Normal G6PD		No	
	No.	%	No.	%	%	
Rh D Positive	35	100	928	95	963	95.2
Rh D Negative	00	00	49	5	49	4.8
Total	35	100	977	100	1012	100

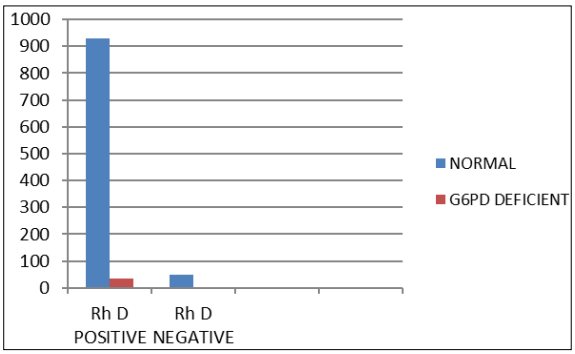

Fig 4: Donors' Rhesus blood group distribution and their G6PD status

Table 5: All positive results of MRT and quantitative test

Sr. no.	Unit no.	MRT result	Quantitative test result	Sr. no.	Unit no.	MRT result	Quantitative test result
1	4613	positive	5.8 U/gHb	19	6625	positive	5.2 U/gHb
2	5866	positive	5.2 U/gHb	20	6653	Positive	6.2 U/gHb
3	5874	positive	5 U/gHb	21	6654	Positive	5.4 U/gHb
4	6229	positive	5.9 U/gHb	22	6656	Positive	5 U/gHb
5	6281	positive	5 U/gHb	23	6672	positive	6.2 U/gHb
6	6331	positive	5 U/gHb	24	6678	positive	4.2 U/gHb
7	6332	positive	4.5 U/gHb	25	6722	positive	3.4 U/gHb
8	6353	positive	3.8 U/gHb	26	6734	positive	4.6 U/gHb
9	6398	positive	3.6 U/gHb	27	8359	positive	5 U/gHb
10	6468	positive	4.1 U/gHb	28	8601	positive	5 U/gHb
11	6473	positive	4.5 U/gHb	29	13414	positive	4.8 U/gHb
12	6490	positive	4.5 U/gHb	30	13441	positive	5.3 U/gHb
13	6496	positive	5.2 U/gHb	31	13457	positive	6.3 U/gHb
14	6520	positive	4.6 U/gHb	32	13514	positive	4.5 U/gHb
15	6539	positive	3.7 U/gHb	33	13679	positive	4.7 U/gHb
16	6550	positive	4.5 U/gHb	34	13652	positive	3.5 U/gHb
17	6571	positive	4.7 U/gHb	35	13624	positive	4.5 U/gHb
18	6617	positive	4.5 U/gHb				

As mentioned in Table 5, all the donors who have been G6PD deficient by the methaemogobin reduction test have been confirmed by quantitative method using semi automated G6PD reagent kit (Pathozyme Diagnostics). Quantitative results of these positive donors were between 3.4 U/gHb to 6.3 U/gHb with mean value of 4.7 U/gHb which were lower than the normal range (6.4 to 20 U/gHb).

Discussion

This study represents the attempt to evaluate the prevalence of G6PD in healthy blood donors of Blood Bank at tertiary care hospital and Government medical college level set up. The results of this study showed that out of total 1012 healthy blood donors, 35(3.5%) blood donors had G6PD deficiency and 977 (96.5%) blood donors had normal G6PD levels.

Table 6: Comparison of various studies for prevalence of G6PD deficiency in blood donors

Study	Year	Country	N	Prevalence
White *et al*. [4]	1986	Yemen	146	6.2%
Choubisa *et al*. [5]	1987	Rajasthan, India	1198	4.59%
Pant *et al*. [6]	1992	Gujarat, India	414	5.9%
Ramadevi *et al*. [7]	1994	South India	5140	7.8%
Kaeda [8]	1995	Orissa, India	49	6.12%
Hilmi *et al*. [9]	2002	Iraq	758	6.1%
Sukumar *et al*. [10]	2004	Mumbai, India	3166	10.5%
Matsuoka *et al*. [11]	2004	Cambodia	670	7.0%
Nishank [12]	2008	Orissa, India	3480	6.41%
Pankajkumar *et al*. [13]	2016	Rajasthan, India	2012	6.6%
Present study	2019	Gujarat, India	1012	3.5%

Table 6 shows the comparison of studies for prevalence of G6PD deficiency in blood donors. The prevalence rate (3.5%) observed in the present study was lower than that of other studies.

All the donors who have been G6PD deficient by the methaemogobin reduction test have been confirmed by quantitative method using semi automated G6PD reagent kit (Pathozyme Diagnostics). Quantitative results of these positive donors for G6PD levels were between 3.4 U/gHb to 6.3 U/gHb with mean value of 4.7 U/gHb which were lower than the normal range (6.4 to 20 U/gHb). As all positive results by methaemoglobin reduction test also showed deficient G6PD values by quantitative method, methemoglobin reduction test can be used for screening of G6PD deficiency.

Overall most common age group was also 21-30 years where total 445 blood donors were found. Second most common age group was 31-40 years where total 341 blood donors were found. Our study is similar to Sidhu *et al*. [14] observed that majority of the blood donors belonged to age group 18-30 years. All the 07 females had normal G6PD level. The reason behind this fact is that the abnormal gene responsible for deficiency is located on the X chromosome. Therefore, the illness associated with G6PD deficiency occurs more frequently in males than in females (Beutler, 1993). The higher rate of male donors than females may be a result of their high packed cell volumes (PCV) which is usually higher than that of their female counterparts. The normal range for the male is between 40 - 54% and that of the females is between 36 - 46%. Besides, higher hemoglobin values in males (normal range 13 - 18 g/dl) compared to females (12 - 15 g/dl) or other screening criteria of the donors usually exclude the females from blood donation.

In our study most common ABO blood group was B blood group which is similar to a study done by Pant *et al*. [6] who observed distribution of ABO blood groups and sickle cell haemoglobin on 783 blood samples in relation to malaria, from both the sexes of Muslim and Christian populations of Kheda district, Gujarat. Our study found that out of total 1012 blood donors, 963(95.2%) blood donors were Rh D Positive and 49(4.8%) blood donors were Rh D Negative. Our study is similar to Sidhu *et al*. [14] who observed that out of total 500 blood donors 445(89%) of blood donors were Rh D Positive and 55(11%) of blood donors were Rh D Negative.

Transfusion with G6PD-deficient blood carries a potential risk of hemolytic complications, especially if it is used for exchanged blood transfusion in neonates. For these reasons, in high-prevalence areas for G6PD deficiency, donation can be accepted but G6PD-deficient blood should be labeled and should not be released to transfuse a G6PD-deficient patient or to exchange in the pediatric age group, particularly neonates. In blood banks with limited resources where screening for SCT and G6PD deficiency is not feasible, we advise to screen the units that are likely to be transfused to high-risk recipients [15].

Conclusion

The observation of 3.5% prevalence of G6PD deficient blood donors in the present study could be taken to consider that the problem of G6PD deficiency exists in the blood donors. It should be of concern as the G6PD deficiency remains obscure, there being no overt clinical manifestation. As the study was conducted over a time period of nine months from March to November 2019 involving 1012 blood donors, prevalence of 3.5% should be taken as serious concern and further more studies are advised for screening of G6PD in healthy blood donors.

As all positive results by methemoglobin reduction test also showed deficient G6PD values by quantitative method, methaemoglobin reduction test can be used for screening of G6PD deficiency. Also the methaemoglobin reduction test (MRT) used in this study is cost effective and convenient, it can be used as a screening method to identify G6PD deficient blood donors and it can be an aid-on extended medical checkup for healthy blood donors which will help to increase the safe and voluntary blood donations.

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