TRANSFUSION MEDICINE

Original article

High prevalence of G6PD mutations in blood donors from the Brazilian Amazon region

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Background - Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a genetic condition caused primarily by single nucleotide polymorphisms (SNPs). It is the most prevalent enzymopathy globally and directly impacts the ability of red blood cells to respond to oxidative stress. This study aimed to determine the prevalence of G6PD variants in Amazon blood donors.

Materials and methods - A cross-sectional study was conducted at the Foundation of Hemotherapy and Hematology of Amazonas (HEMOAM) between January and June of 2024. The study analyzed 5,000 donor samples using the quantitative polymerase chain reaction (qPCR) to detect G6PD genetic variants.

Results - G6PD variants were present in 364 (7.28%) donors, with the most common being c.376A>G (3.82%), c.292G>A (1.92%), and simultaneous c.292G>A/376A>G (0.90%). Other rare variants were also identified, including c.653C>T, c.1093G>A, c.968T>C; c.632A>T; c.1429G>A; c.700G>T; c.1192G>A and c.197T>A/c.202G>A, representing 0.64% of the total. Most donors exhibiting mutations were male (71.7%), with a mean age of 34.1±11.9 years for males and 34.0±10.5 years for females. The most prevalent blood group in both sexes was O+, with a frequency of 59.7% in males and 61.9% in females.

Discussion - This study constitutes the most extensive molecular testing for G6PD mutations in the state of Amazonas, offering a comprehensive overview of G6PD deficiency (G6PDd) in the region. The estimated prevalence rate of G6PD mutations among Manaus blood donors was 7.28%, which is consistent with other findings in Brazil. In conclusion, G6PDd has the potential to compromise red blood cell quality during storage and their survival post-transfusion. This study enhanced the methods used to identify mutations impacting blood storage, with the aim of improving transfusion safety and efficacy.

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Keywords: *G6PD variants, blood transfusion, blood donors, Amazon, Brazil.*

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PDd) is caused by single nucleotide polymorphisms (SNPs) linked to the X chromosome. Approximately 5% of the world's population, or about 400 million people, suffer from this condition[1]. G6PD is a ubiquitous enzyme involved in detoxifying oxidants. In erythrocytes, its primary function

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is to protect against oxidative stress by providing reduced nicotinamide adenine dinucleotide phosphate (NADPH) to regenerate reduced glutathione (GSH), thereby counteracting increases in reactive oxygen species (ROS) present in this environment[2].

Due to G6PD's X-linked inheritance, males are more affected as they are hemizygous for mutations, while females may be heterozygous or homozygous. Although heterozygous individuals generally do not manifest the deficiency, they may still exhibit it depending on the mosaicism of their somatic cells[3]. G6PD mutations were originally classified in 1971 and variants grouped into five classes (I to V) based on enzyme activity and clinical severity[4]. Class I, the most severe, was associated with chronic non-spherocytic hemolytic anemia (CNSHA) and very low enzyme activity (<1%). Class V included variants with increased enzyme activity (>150%) and no clinical symptoms. Classes II, III, and IV represent varying degrees of deficiency, with symptoms ranging from moderate to mild in class II, having less than 10% enzyme activity and a risk of acute hemolytic anemia, while Class III and IV involved moderate activity (10-60%) and mild to normal activity (60-150%), respectively, the latter typically being asymptomatic[4].

The World Health Organization (WHO) updated the G6PD classification in 2022, streamlining it into four categories: A, B, C, and U[5]. Categories A and B correspond to variants with reduced enzyme activity, reflecting the severity of classes I, II, and III. Class C includes variants with normal or increased enzyme activity, similar to classes IV and V, which were typically asymptomatic. A new addition, designated as "Class U," was created for variants exhibiting enzyme activity outside the standard range, or whose clinical significance is unknown or uncertain[5]. This updated system provides a clearer, more concise framework for understanding the clinical implications of G6PD mutations[6].

In Brazil, several studies showed a highly variable prevalence of G6PDd, with rates ranging from 1.7% to 24%, depending on the region analyzed. The northern and northeastern regions of Brazil were documented as areas of high prevalence of G6PDd. Notably, the state of Amazonas was observed to have particularly elevated rates of this condition, particularly among blood donors[7-9]. The single nucleotide variants (SNVs) most frequently linked to diminished enzyme activity in Brazil are predominantly associated with African A– (old classification: c.202G>A/c.376A>G; new: c.292G>A/c.376A>G) and Med (old classification: c.563C>T; new: c.653C>T) origins, with a combined estimated prevalence exceeding 95% in Brazilian investigations. Research indicates the presence of three distinct ethnic groups constituting the Brazilian populace: the original inhabitants (indigenous), who were added to by Europeans (primarily Portuguese) and Africans (predominantly from the western coast, south of the Sahara). These data substantiate the profound genetic heterogeneity of Brazil, which was influenced by historical immigration, particularly from Africa after the 15th century[10-12].

Currently, there are ongoing debates and discussions regarding transfusion medicine, specifically how long stored red cells maintain their efficacy as a viable product. Techniques for storing and transfusing packed red blood cells may increase oxidative stress, which may reduce erythrocyte survival in people with G6PDd[13-15]. In addition, the WHO recommends implementing guidelines for evaluating potential blood donors in areas with a high prevalence of G6PDd. As examples, red blood cells from donors with G6PDd should not be used for intrauterine transfusions, neonatal exchange transfusions, and patients with G6PDd. Moreover, individuals with G6PDd who have experienced hemolysis should be permanently excluded from donating blood, according to WHO guidelines[16-18]. Despite these recommendations, blood donors are not routinely screened for G6PDd.

Considering the existing research that already demonstrated a high incidence of G6PDd in the northern region of Brazil, specifically within the state of Amazonas, the objective of the current study was to ascertain the prevalence of the primary genetic variants of G6PD that have already been documented in the country, utilizing a representative population and a nucleic acid amplification test (NAT) that has already been implemented in the local blood center[19].

MATERIALS AND METHODS

Study design

This was a cross-sectional study conducted on blood donors from the Amazonas Hemotherapy and Hematology Foundation (HEMOAM) during the period from January to June 2024.

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Context

The Foundation for Hemotherapy and Hematology of Amazonas is a preeminent public hematology center in the state of Amazonas, located in Manaus, the largest state capital in Brazil with a population of nearly 2,600,000. The Foundation plays a pivotal role in providing blood and hematology services to the local community, supporting both routine and emergency medical needs. The Foundation is instrumental in ensuring a stable supply of blood products and advanced hematology care, thereby making a significant contribution to public health in the region.

Study population and sampling

The present study focused on blood donor candidates at HEMOAM. For the detection of G6PD SNVs, the same genetic material routinely extracted for use in NAT was utilized. Surplus genetic material from NAT (approximately 15-20 µL) was aliquoted into 96-well plates. In some cases, samples were pooled from six donors, while in other cases, they were processed individually. In instances where wells containing six samples exhibited positive results, individual blood samples were then subjected to new DNA extraction for subsequent individual sample analysis.

The sampling plan was designed to span a period of four weeks, with random samples collected from January to June of 2024. Based on an average of 250 blood donations received daily at HEMOAM, and considering collections from Monday to Friday each week, a minimum total sample size of 5,000 was calculated. This number is derived from approximately 1,250 weekly donations at HEMOAM.

Molecular analyses

Molecular testing was performed after completing the daily NAT routine. All samples, including those from the mini-pool, were measured for concentration using the NanoDrop™ 2000 UV visible spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). They were then analyzed in triplicate using the StepOnePlus™ Real-Time PCR System, 7500 Fast Real-Time PCR, and QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Waltham, Massachusetts, USA). The G6PD genetic variants studied were selected based on their known frequencies in Brazil and their clinical significance according to the 2018 WHO guidelines. The specific probes used to amplify each polymorphism are (old/new classification): c.202G>A/c.292G>A (rs1050828); c.376A>G (rs1050829); c.542A>T/c.632A>T (rs5030872); c.563C>T/c.653C>T (rs5030868); c.680T>C/c.770T>C (rs76723693); c.1003G>A/c.1093G>A (rs5030869); c.1192G>A (rs137852325) and c.1339G>A/c.1429G>A (rs137852317).

Variant confirmation

Samples testing positive for any variant were validated by gene sequencing (Sanger, 1977) using the 3500 Series Genetic Analyzer (Thermo Fisher) and DNASTAR software (NCBI Reference Sequence: NM_000402.4)[20].

Data collection

Data were collected from individuals whose samples were included in the study using the HemoSIS platform (HEMOAM, Manaus, Brazil). Data included age, gender, race, municipality, education level, hematocrit, blood group, and number of donations. Additionally, these data allowed for individual monitoring to prevent duplicate entries in the study.

Statistical analyses

The data were examined for consistency with the results of the molecular and demographic data sets, cleaned of potential double data entry. This was accomplished using Excel software (Microsoft, New York, NY, USA). Subsequent analysis of the refined data set used the Statistical Package for Social Sciences, version 22 (SPSS Statistics, Chicago, IL, USA), contingent upon the nature of the variable in question.

RESULTS

A total of 5,000 samples were submitted for genetic analysis, resulting in the identification of SNVs associated with G6PDd in 364 (7.28%) blood donors, most of whom were male (71.7%). The study confirmed African ancestry as the most prevalent, with the G6PD A+ (3.82%), G6PD Asahi/A– (1.92%), G6PD A– (0.9%), and G6PD A– (Nefza) variants comprising 91.21% of all SNVs identified, while Mediterranean was the second most prevalent ancestry, comprising 2.47% of SNVs. It is important to note that some SNVs can be co-inherited, with an individual carrying more than one variant. Our study identified co-inheritance in 45 (0.9%) donors carrying the G6PD A– variant (c.292G>A/c.376A>G), and two (0.04%) with the SNVs (c.287T>A/c.292G>A), corresponding to co-inheritance in 0.94% of donors. Three donors were

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Table I - *Frequency of G6PD variants in blood donors from HEMOAM*

Variant	SNV ID old mutation site	HGVS old nomenclature	HGVS new nomenclature	Amino acid change	Class		No.	G6PD activity (clinical symptoms)	Prevalence this study (%)
					1985	2022			
G6PD A+	rs1050829	c.376 A>G		p.Asn126Asp	III	C	191	>60% (NH)	3.82
G6PD A– (Asahi)	rs1050828	c.202 G>A	c.292 G>A	p.Val98Met	III	B	96	<45% (AHA*)	1.92
G6PD A–	rs1050828/ rs1050829	c.202 G>A/ c.376 A>G	c.292 G>A/ c.376 A>G	p.Val98Met/ p.Asn126Asp	III	B	45	<45% (AHA*)	0.90
Mediterranean	rs5030868	c.563 C>T	c.653 C>T	p.Ser218Phe	II	A	9	<20% (CHA)	0.18
Chatham	rs5030869	c.1003 G>A	c.1093 G>A	p.Ala365Thr	II	B	8	<45% (AHA*)	0.16
G6PD A– (Nefza)	rs76723693	c.968 T>C		p.Leu323Pro	III	B	5	<45% (AHA*)	0.10
Santa-Maria	rs5030872	c.542 A>T	c.632 A>T	p.Asp211Val	II	B	4	<45% (AHA*)	0.08
Santiago de Cuba	rs137852317	c.1339 G>A	c.1429 G>A	p.Gly477Arg	I	A	2	<20% (CHA)	0.04
G6PD Mexico City	rs137852328	c.680 G>T	c.770 G>A	p.Arg257Gln	III	B	1	<45% (AHA*)	0.02
G6PD Puerto Limón	rs137852325	c.1192 G>A		p.Glu398Lys	I	A	1	<20% (CHA)	0.02
G6PD Bahia	**/rs1050828	c.197T>A/ c.202G>A	c.287T>A/ c.292G>A	p.Phe66Tyr/ p.Val98Met	III	B	2	<45% (US***)	0.04
Total	Positive for some SNV						364		7.28
	Wild Type						4,636		92.72

SNV ID: single nucleotide variant number identification; No.: total number sample; class: previous (1985) and current WHO classification of G6PD variants (2022)[5]; CHA: chronic hemolytic anemia; AHA: acute hemolytic anemia*; NH: no hemolysis; US: uncertain significance. (G6PD activity/clinical symptoms): adapted from WHO (2022)[5] and Jin *et al.* (2024)[18]. *Anemia triggered by certain medicines, fava beans or infection. **It was only possible to find SNV (c.197) through DNA Sanger sequencing[20]. ***Given its co-inheritance with c.292G>A, it is possible for it to manifest as AHA. HGVS: Human Genome Variation Society (De Dunnen *et al*., 2016[50]). Adapted by https://www.snpedia.com/index.php/G6PD. The variants have been uploaded in the Leiden Open Variation Database for G6PD https://databases.lovd.nl/shared/genes/G6PD.

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identified as carriers of G6PDd currently classified as A (Mediterranean, Mexico City, and Puerto Limón), which are reported as Chronic Hemolytic Anemia due to the enzymatic activity of G6PD being less than 20% of normal (**Table I**).

The mean age of male donors was 34.1 years (±11.9) and of female donors was 34.0 years (±10.5). A statistically significant difference was observed between sexes for hematocrit value (Hct) ($p<0.001$), with males exhibiting values of 43.2±2.6 and females exhibiting values of 39.7 ±1.6 (*data not shown*). The most prevalent blood group was O+, followed by A+, B+, and AB+; these trends were observed in both male and female subjects. Group O constituted 65.86% of all blood donors, while group AB constituted only 1.56% (**Table II**).

A quantitative analysis of blood bags received from blood donors residing in cities within the state of Amazonas is presented in **Table III**. The blood bags were categorized by sex, and the data analyzed by NAT-HEMOAM. As expected, Manaus, being the capital of Amazonas, accounts for most blood donors (88.24%), while São Gabriel da Cachoeira represents only 1.48%. For reasons that remain unclear, no female samples were collected from Tefé during the collection period.

Table II - *Distribution of ABO blood groups among HEMOAM blood donors, categorized by sex*

ABO blood group		Male		Female		Total
		No.	%	No.	%	
O	Rh +	2,154	60.1	874	61.8	3,028
	Rh -	192	5.4	73	5.2	265
A	Rh +	815	22.7	285	20.1	1,100
	Rh -	102	2.9	52	3.7	154
B	Rh +	272	7.6	95	6.7	367
	Rh -	4	0.1	4	0.3	8
AB	Rh +	42	1.2	30	2.1	72
	Rh -	4	0.1	2	0.1	6
Total		3,585	100.0	1,415	100.0	5,000

No.: total number sample.

Table III - *Number of RBC units obtained from blood donors residing in Amazonas State cities, categorized by sex, analyzed by NAT-HEMOAM*

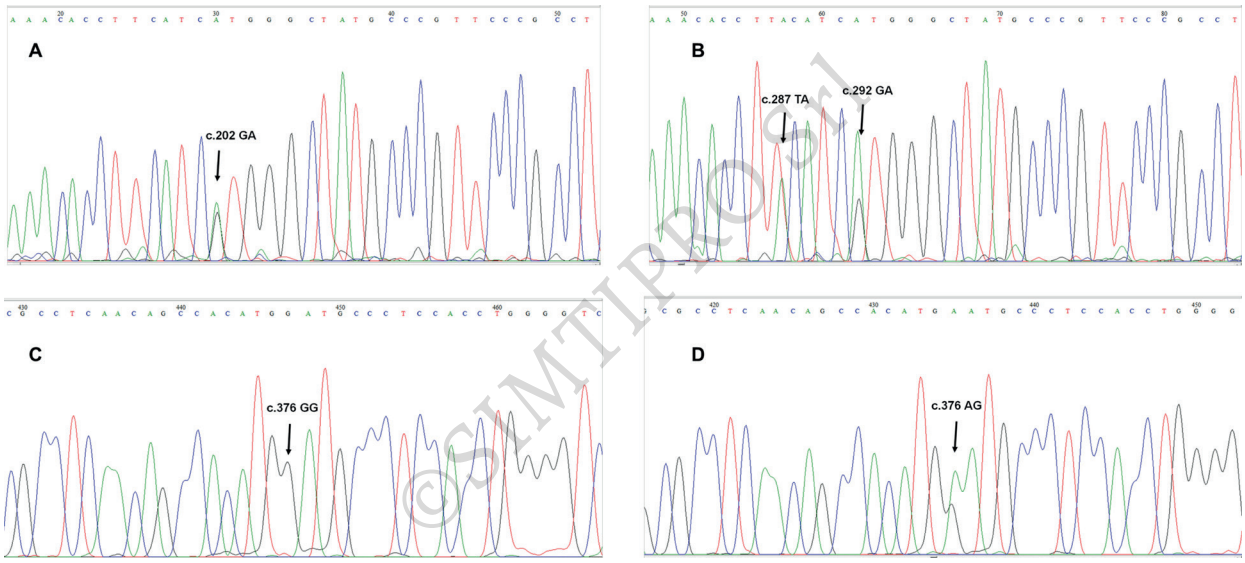
Cities	Male		Female		Total
	No.	%	No.	%	No.
Manaus	3,118	87.0	1294	91.4	4,412
Coari	115	3.2	10	0.7	125
Itacoatiara	75	2.1	21	1.5	96
Parintins	65	1.8	52	3.7	117
Manacapuru	54	1.5	7	0.5	61
São Gabriel da Cachoeira	43	1.2	31	2.2	74
Tefé	115	3.2	0	0	115
Total	3,585	100.0	1,415	100.0	5,000

No.: total number sample; RBC: red blood cell.

Table IV - *Categorization of race and ethnicity according to sex distribution among HEMOAM blood donors*

Race/Ethnicity	Male		Female		Total
	No.	%	No.	%	No.
Brown	2,670	74.5	1,098	77.6	3,768
White	265	7.4	127	9.0	392
Indigenous	158	4.4	31	2.2	189
Black	158	4.4	21	1.5	179
Yellow	11	0.3	10	0.7	21
Didn't know how to answer	323	9.0	128	9.0	451
Total	3,585	100.0	1,415	100.0	5,000

No.: total number samples



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Figure 1 - Electropherograms illustrating the most prevalent G6PD variants identified and validated in this study
Panels **A**, **B**, and **D** illustrate heterozygous genotypes of the African variants c.292GA and c.376AG in female donors, while panel **C** displays a male donor with the hemizygous genotype of the c.376GG variant. Panel **B** also demonstrates the concomitant presence of a heterozygous genotype comprised of the c.287TA and c.292GA variants.

In accordance with previous work, the predominant proportion of the donor population self-identified as "brown" (75.36%), with smaller percentages identifying as white, black, yellow, and Indigenous. Within the Brazilian context, the designation "brown" pertains to individuals considered to be of mixed ancestry, encompassing European, African, and indigenous lineage. In contrast, "white" denotes individuals predominantly of European and North American ancestry, while "black" signifies those of African descent and "yellow" refers to individuals of East Asian ancestry (**Table IV**)[13-15].

In this study, the most prevalent G6PD variants (SNVs c.292G>A and c.376A>G) were validated through DNA sequencing [20]. It is noteworthy that DNA sequencing was instrumental in identifying and validating the presence of the G6PD c.197T>A (current c.287T>A) variant. As initially described in the state of Bahia, Brazil, it was only found in combination with c.202A>G, which is known to contribute to G6PDd (ClinVar ID 37123) (**Figure 1**)[9].

DISCUSSION

The primary G6PD variants identified in blood donors at HEMOAM were of African origin followed by those of Mediterranean origin. Our results corroborate several other Brazilian and Latin American studies[7,8,21-24].

The prevalence of G6PD variants varies widely across different regions of Brazil. The South and Southeast regions, historically influenced by European, Asian, and African colonization, report prevalence rates of 1.7-10.4%. While most variants are of African origin, these regions also show a comparatively higher frequency of Mediterranean and European variants[25,26].

A study conducted of newborns in the Central-West region of Brazil, using both enzymatic activity and molecular genotyping, detected the G6PDd in three major cities. The study reported G6PDd prevalence of 1.67% Cuiabá (31/1857), 2.12% (20/945) Várzea Grande, and 1.56% (12/771) in Rondonópolis, providing an average of 1.78% of G6PDd in the region. Molecular genotyping demonstrated the presence of SNV c.202G>A in 92% of carriers of G6PDd, and no newborn presented with SNV c.563C>T[27].

The Northeastern region of Brazil was identified as having one of the highest mean G6PDd prevalence rates, ranging from 2% to 11.86%. As in other regions of Brazil, the main variants were of African origin[28,29]. Notably, a novel variant, termed "Bahia (c.197 T>A)," was identified in neonates in the State of Bahia, with a prevalence of 0.76% (5/655). In this study, SNV c.197T>A was always present concomitantly with the SNVs c.202G>A and c.376A>G. We observed a similar finding, where SNV c.197T>A in two donors was concomitantly present with the SNV c.292G>A (old c.202G>A)[9].

The North region is the most studied in relation to G6PDd, due to endemic malaria. In the literature, G6PDd has been widely associated with malaria, with studies both supporting and challenging the idea that this enzyme deficiency may protect against malaria infection[30,31]. The most prevalent variants in this region are of African origin. In the state of Pará, two studies reported prevalence rates of 5.7-24%[8,32]. The states of Amapá and Roraima had prevalence rates of 5.8% and 4.2%, respectively. In Acre, prevalence rates ranged from 3.9% to 8.9%, while in Rondônia, rates varied from 3.6% to 6.3%, depending on the municipality investigated[32,34].

Finally, in the Amazon, the Brazilian state where this study was conducted, Barraviera *et al*. was among the first to report a G6PDd prevalence of 4.9% in a cohort of 141 individuals from the municipality of Humaitá, a rural community in Amazonas[35]. Subsequently, Santana *et al*. demonstrated a 3% prevalence of G6PDd in a cohort of 200 men from the malaria-endemic rural community of Ismael Aziz[36]. In a later study, the same authors reported a G6PDd prevalence of 4.5% among 1,478 male patients with Plasmodium vivax malaria, with 3.8% exhibiting the c.202G>A and c.376A>G mutations, and 0.7% exhibiting the c.563C>T mutation[37].

Several studies revealed significant variation in the prevalence of G6PD variants among different populations in Manaus. One study of post-mortem *P. vivax* malaria patients identified hemorrhagic conditions in two cases following primaquine use, confirming the c.202G>A and c.376A>G variants[38]; a similar study in the Manaus area, using molecular methodology, found a G6PDd prevalence of 12.9%, with all patients having the African A- (c.202G>A) and A+ (c.376 A>G) variants[39]. At the Hematology and Hemotherapy Foundation of Amazonas, a G6PDd prevalence of 8.26% was identified among 1,222 blood donors, with 7.7% carrying the c.202G>A and c.376A>G mutations, with rare variants, such as Mediterranean (0.25%) and Chatham (0.08%), detected[7]. In another study of *P. vivax* malaria patients from Manaus-AM, 12.88% carried the c.202G>A mutation, while 19.11% had the c.376A>G mutation[40]. A 2024 study on 157 acute myeloid leukemia patients treated at HEMOAM found G6PD polymorphisms in 18 individuals, including c.202G>A in 8 (5.1%), c.376A>G in 18 (11.5%), and both mutations in 4 (2.5%)[41]. Despite the numerous studies on G6PD in the Amazon region, no previously published research linked G6PD mutations to blood donors with such a large sample size, and with a methodology that uses surplus samples, like the current protocol.

In this study, two variants (c.632A>T and c.770G>T) were identified, which were previously reported in the literature as c.542A>T and c.680G>T, respectively[42]. These variants were classified as Class II (Class A) and Class III (Class B). Furthermore, two rare Class I (Class A) variants (Santiago de Cuba and Puerto-Limón) were identified, which, according to the literature, are associated with severe G6PDd[43,44]. Individuals with these

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variants typically present with Chronic Non-Spherocytic Anemia, characterized by symptoms of chronic anemia and hemolysis. Consequently, these individuals are generally disqualified from blood donation during clinical screening[45]. The approval of these three women for blood donation can be attributed to the heterozygous status of their mutations, possibly combined with mosaicism in their somatic cells. As observed in heterozygous women, some produce a higher proportion of normal erythrocytes, while others produce a predominance of G6PDd erythrocytes. It is likely that the women in this study belong to the former group, which may have prevented the manifestation of this condition[3].

Although the prevalence of G6PD variants varies greatly in Brazil and worldwide, of the nine variants found in this study, three (c.292G>A, c.376A>G, and c.653C>T) accounted for more than 98% of those detected. Of these, only the 292G>A and 653C>T, currently both class B, and previously as class III and II, respectively, can cause hemolysis when exposed to high levels of oxidative stress. The 376A>G variant, when alone, does not induce hemolysis, even in conditions of high oxidative stress. It is noteworthy that the other solo variants identified in 30 donors (c.1093G>A, c.968T>C, c.632A>T, c.1429G>A, c.770G>A and c.1192G>A), as well as c.292G>A and c.376A>G, exclusively induce hemolysis in the presence of elevated levels of oxidative stress. However, reducing oxidative stress, even with the diminished enzyme activity in these variants, is sufficient to stop hemolysis[46-48].

The prevalence of G6PD SNVs among the inhabitants of the Manaus region is quite high, which is also reflected in the blood donors in this area. Implementing screening for G6PD variants at the time of donation is a prudent measure to enhance the safety of the blood products provided by the regional blood bank. We believe that elucidating the enzymatic activity of the respective variants found may help provide genetic counseling and prevent the occurrence of complications, especially in recipients of transfused red cells from donors carrying variants for G6PDd, as well as for developing strategies to improve the storage quality of the red cells obtained from these donors.

The methods employed in this study to detect genetic variants associated with G6PDd have two major implications. First, they expand the possibility of performing genetic tests to screen blood donors. Second, they establish an efficient alternative to conventional methods[49]. In summary, these methods could provide faster, more accurate, and more reliable detection of other important genetic variants relevant to blood donation, storage, preservation, and transfusion.

Limitations

Due to the cross-sectional design of the study, it was not possible to ascertain causality; only frequency data were analyzed. Furthermore, the genetic data exclusively encompass the specific variants targeted by the selected probes, which may preclude the consideration of other genetic variations in different regions of the G6PD gene. Finally, given the nature of the study design, G6PD enzymatic activity was not quantified for any of the donated red blood cell units.

ETHICAL CONSIDERATION

The research project has already been approved by the Research Ethics Committee (CEP) of HEMOAM in Resolution 466/2012, CAAE: 70959223.4.0000.0009/ Number: 6.325.722.

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AUTHORS' CONTRIBUTIONS

JNVS, JPMN, SRLA conceived and designed the initial survey. JNVS, JPMN, MMS contributed to the practical

laboratory work. SRLA acquired blood donor data from the system. Other Authors contributed with technical adjustments and writing support for this article

The Authors declare no conflicts of interest.

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