ORIGINAL ARTICLE

THE PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN HEALTHY BLOOD DONORS IN KARACHI, PAKISTAN: A MALARIA ENDEMIC AREA

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ABSTRACT

Background: Annually 3.5 million presumed and confirmed significant cases of malaria are reported in Pakistan. The hypnozoite stage of *Plasmodium vivax* can be eradicated via antimalarial i.e. Primaquine, and this drug causes hemolysis in G6PD enzyme deficient individuals. Therefore, it is important to map malaria distribution and G6PD deficiency in malaria endemic area i.e., Karachi Pakistan to design an effective malaria eradication regimen.

Material and Methods: Adult healthy male blood donors, were recruited after informed consent during 1st March 2018 till 30th November 2020 in a hospital blood bank at Karachi, Southern Pakistan. G6PD enzyme was measured by a qualitative method (Trinity Biotech Glucose-6-Phosphate Dehydrogenase Qualitative Kit catalogue no. 400-10*10, Sweden) based on detecting the rate of reduction of NADP to NADPH through decolourization of dye. Tested individuals were classified as G6PD-normal, -intermediately deficient, and –completely deficient based on color change within the time frame of 20, 20-60 and after 60 minutes.

Results: 29 (8.4%) out of 342 adult male blood donors were identified to be G6PD deficient. 28 (8.1%) were intermediately deficient for G6PD deficiency while one was completely deficient (0.3%).

Conclusion: Our small-scale study indicated an appreciable frequency of G6PD deficiency in Karachi Pakistan, requiring mandatory G6PD screening prior to administration of primaquine for malaria eradication. Large-scale studies are required for complete mapping of the city which will eventually build into malaria-roll-out program.

Keywords: Deficiencies, Glucose phosphate dehydrogenase, G6PD deficiency, Blood donors, Anemia, Hemolysis, Malaria

BACKGROUND

Malaria continues to be a serious public health concern in Pakistan and leads to life threatening infection if not treated.[1] According to World Malaria Report (2019), 28.9% Pakistani population lives in high transmission, 69.4% in low and medium, and 1.7% in no transmission risk areas with around 72 districts/territories in Balochistan, Khyber Pakhtoonkhwa and Sindh shared major malaria case load, whereas Punjab, Azad Jammu and Kashmir, Islamabad and Gilgit Baltistan are among least affected areas.[2] In Pakistan, like other Asian countries, malaria is caused predominantly (88%) by *Plasmodium vivax, and Plasmodium falciparum* infection is observed mostly in rain or post-rain season.[(1)] The latent hypnozoite stage of *P. vivax* in liver of infected individuals causes relapse events for months or years following the early infection which can be prevented by antimalarial.[3] Primaquine, 8-aminoquinoline anti-malarial, is used to treat and eradicate hypnozoite stage but may cause hemolysis (methemoglobinemia) in Glucose-6-phosphate dehydrogenase (G6PD) deficient patients.[4]

G6PD is the commonest red blood cell enzyme, that protects red cells against oxidative damage.[5,6] The deficiency of G6PD affects 400 million people globally and represents about 8% of people living in malaria endemic countries.[5,6] The clinical phenotypes of G6PD deficiency are variable ranging from completely asymptomatic to severe life-threatening hemolysis triggered by drugs, infections etc.[7] This means that patients will continue to suffer morbidity and *P.vivax* elimination will never happen unless primaquine or other anti-hypnozoite is given. Hence, it mandates that detection of G6PD levels is extremely important for malaria patients, before planning to prescribe them primaquine. Also, on the basis of carrying one or two

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copies of the defective gene, it is important to note that phenotypically, females are either normal, deficient (homozygous) or carrier (heterozygous), while males are either normal or deficient.[3]

A recent meta-analysis showed a global prevalence of 4.5% G6PD deficiency with an overall incidence of 1.8% in Pakistan, the highest prevalence reported in Northern areas of up to 8%.[5,6] No estimates are available for Southern Pakistan.Karachi is the largest and one of the most populous cities of Pakistan. Moreover, it's a highly endemic area for malaria with seasonal peaks.[8] Therefore, it is important to map malaria distribution and G6PD deficiency in Karachi to design an effective malaria eradication regimen.[9] Annually, 3.5 million presumed and confirmed significant cases of malaria are reported in Pakistan and the next vision of Malaria Control program is to eliminate Malaria from Pakistan till 2023.[10]

With this intent of eradicating malaria from Pakistan till 2023, we aim to screen healthy blood donors for G6PD levels, so that prevalence of its deficiency is known and we can safely plan to give antimalarial i.e., primaquine without the fear of hemolysis. Therefore, this study shall provide a database for future studies that may help in planning malaria eradication in local setting.

MATERIAL AND METHODS

In this cross-sectional study, adult healthy male blood donors 18-60 year of age, were recruited from Blood Bank, Aga Khan University Hospital from 1st March 2018 till 30th November 2020.Enrolled subjects were apparently healthy, lacked any history of malaria in last three years, jaundice, or chronic hemolytic anemia and were not on any medication at the time of blood sampling. Female blood donors and those belonging to other countries were excluded. Data was collected and results were recorded on an in-house proforma.

Hemoglobin was analyzed by the point of care testing device (HemoCue® Hb 201$^+$ Sweden) as mandatory testing for donor selection. Four ml whole blood sample was collected from each enrolled subject for G6PD qualitative screening (Trinity biotech G6PD kit catalogue no. 400-10*10, Sweden) which is based on the principle of dye decolourization when NADP is reduced to NADPH in the presence of G6PDH. Depending on the rate of color change (<20, 20-60, >60 minutes), an individual is considered as G6PD-normal, intermediate deficient or complete deficient. Internal quality controls for both tests were used as per manufacture's recommendations.

The data was analyzed on Statistical Package for the Social Sciences (SPSS, IBM, version 20). Mean ±SD was used for age and hemoglobin estimation. Frequency and percentages were computed for G6PD deficiency in healthy blood donors.

The study was approved by ethical review committee of Aga Khan University Hospital, Karachi (#5042-Pat-ERC-17) and written informed consent was obtained from each participant. G6PD-intermediate and deficient individuals were informed of their results and counseled for enzymopathy.

RESULTS

A total of 342 healthy male blood donors were enrolled. Mean (±SD) age was 29.89 years ± 7.89 (range: 18-55 years) and mean (±SD) hemoglobin was 14.7 g/dL ± 0.9 (range: 12.5-16.9 g/dL). Of 342 donors, 313(91.5%) were G6PD-normal, 28(8.1%) were G6PD-intermediate deficient and 1(0.3%) was G6PD-complete deficient. Therefore, overall frequency for G6PD deficiency in our study was 8.4% (Table-1).

Table-1: G6PD screening results in 342 healthy blood donors in Karachi, Pakistan.

	N (%)	Age (years) Mean ±SD	Hemoglobin (g/dL) Mean ±SD	G6PD-normal N (%)	G6PD-partial deficient N (%)	G6PD-deficient N (%)
Total	342 (100)	29.89 ± 7.89	14.7 ± 0.9	313 (91.5)	28 (8.1)	1 (0.3)

DISCUSSION

In Pakistan, the provinces of Balochistan, Sindh and Khyber Pakhtunkhwa and the Federally Administered Tribal Areas have the highest malaria burden with predominant infection caused by *Plasmodium vivax*.[11] The *Plasmodium vivax* carries a silent hypnozoite reservoir that cannot be diagnosed until it generates a clinical attack.[12] Primaquine, is regarded as a "Radical

Cure" for reducing malaria burden.[13] But it causes hemolysis in people suffering from G6PD enzyme deficiency. Hence, determining prevalence of G6PD is very important in malaria endemic areas e.g., Karachi, Pakistan. Our small-scale study indicated an appreciable frequency of G6PD deficiency in this malaria endemic area.

Several studies in the past were performed to detect prevalence of G6PD deficiency in various parts of Pakistan. One of the oldest studies was performed by McCurdy *et al*. (1970) in Rawalpindi on 221 subjects, and reported G6PD deficiency in 2.8% individuals.[14] Bollinger *et al*. (1986) in Lahore performed study on 780 subjects and found G6PD deficiency in 2.5%.[15] In our study, we took 342 healthy male blood donors in Karachi and overall prevalence of G6PD deficiency determined was 8.4%. A global prevalence of 4.5% G6PD deficiency was reported in a meta-analysis with an incidence of 1.8% in Pakistan.[16] Ronald *et al*. reported the overall incidence of G6PD deficiency in Pakistani males to be 2.6%.[17] To the best of our knowledge, this is the first study regarding the prevalence of G6PD deficiency in healthy blood donors in Southern Pakistan; a malaria endemic area.

We recruited healthy blood donors in our study for the determination of G6PD deficiency prevalence as this population denotes asymptomatic individuals, with diverse ethnic representation, who have variable G6PD enzyme level without giving any history of malaria or hemolysis.

In our study, the prevalence of G6PD deficiency was 8.4% which emphasizes the need for more screening tests for G6PD deficiency be done in Southern Pakistan for confirmation of our estimate. None of our intermediate and complete deficient donors showed evidence of hemolysis in past and all had hemoglobin ranging from 12.5-16.9 g/dL. The donor who was completely G6PD deficient had hemoglobin of 13 g/dL. In contrast, Khan *et al*. (2004) reported G6PD deficiency in 3.4% adult patients with mild to severe anemia and jaundice in 73% individuals.[18] This difference in symptoms in our study might be related to G6PD levels as majority had intermediate deficiency. Also, none of our G6PD deficient blood donors gave history of drug exposure to antimalarials. The common G6PD deficient variant in Pakistan is "G6PD Mediterranean" with severely low levels of G6PD enzymes.[18] We did not do genetic analysis in this study and it is likely that the study subjects had some other G6PD variant with milder enzyme deficiency.

G6PD deficiency has a heterogeneous distribution and primaquine would be valuable for malaria control and elimination.[9] Hence, knowing the prevalence of G6PD deficiency is very essential for eradication of malaria in our population.

The limitations of this study are being single centre, a small sample size and exclusion of female individuals. Female blood donors are usually carriers for G6PD deficiency and very rarely donate blood as females usually in Pakistan are anemic hence this also needs a larger scale study for determining their G6PD status especially quantitatively. The testing done in our study was qualitative and G6PD enzymatic activity was not quantified in male blood donors.

CONCLUSION

The prevalence of G6PD deficient healthy male blood donors in malaria endemic area i.e., Karachi, Pakistan is 8.4%. Keeping in view its significant percentage, it becomes necessary to screen G6PD levels prior to administration of primaquine for malaria eradication. Further studies should be conducted for its confirmation.

REFERENCES

1. Khattak AA, Venkatesan M, Nadeem MF, Satti HS, Yaqoob A, Strauss K, *et al*. Prevalence and distribution of human plasmodium infection in Pakistan. Malar J 2013;12(1):1-8. DOI: https://doi.org/10.1186/1475-2875-12-297
2. Organization WH. World malaria report. 2020.
3. Allahverdiyev AM, Bagirova M, Elcicek S, Koc RC, Ates SC, Baydar SY, *et al*. Glucose-6-phosphate dehydrogenase deficiency and malaria: a method to detect primaquine-induced hemolysis in vitro. Dehydrogenases. 2012:chapt. 4.
4. Chu CS, Bancone G, Nosten F, White NJ, Luzzatto L. Primaquine-induced haemolysis in females heterozygous for G6PD deficiency. Malar J 2018;17(1):101. DOI:doi.org/10.1186/s12936-018-2248-y
5. Moiz B. A review of G6PD deficiency in Pakistani perspective. J Pak Med Assoc 2013; 63(4): 501-3.
6. Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, *et al*. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A geostatistical model-based map. PLoS Med 2012; 9(11): e1001339.

DOI: 10.1371/journal.pmed.1001339
7. WHO Working Group. Glucose-6-phosphate dehydrogenase deficiency. Bulletin of the World Health Organization 1989; 67(6): 601-11.
8. Ali N, Anwar M, Ayyub M, Bhatti FA, Nadeem M, Nadeem A. Frequency of glucose-6-phosphate dehydrogenase deficiency in some ethnic groups of Pakistan. JColl PhysiciansSurgPak. 2005;15(3):137-41.
9. Moiz B, Arshad HM, Raheem A, Hayat H, Karim Ghanchi N, Beg MA. Frequency of G6PD mediterranean in individuals with and without malaria in Southern Pakistan. Malar J 2017; 16(1): 426. DOI: 10.1186/s12936-017-2069-4
10. Program DoMc. Malaria report 2018. http://dmc.gov.pk/.
11. Kakar Q, Khan M, Bile K. Malaria control in Pakistan: New tools at hand but challenging epidemiological realities.Eastern Mediterranean Health J 2010;16: 54-60.
12. Baird JK, Battle KE, Howes RE. Primaquine ineligibility in anti-relapse therapy of plasmodium vivax malaria: The problem of G6PD deficiency and cytochrome P-450 2D6 polymorphisms. Malar J 2018; 17(1): 42. DOI: https://doi.org/10.1186/s12936-018-2190-z
13. Domingo GJ, Satyagraha AW, Anvikar A, Baird K, Bancone G, Bansil P, *et al*. G6PD testing in support of treatment and elimination of malaria: recommendations for evaluation of G6PD tests. Malar J 2013; 12(1): 391. DOI:doi.org/10.1186/1475-2875-12-391
14. McCurdy PR, Mahmood L. Red cell glucose-6-phosphate dehydrogenase deficiency in Pakistan. J Lab Clin Med 1970; 76(6): 943-48.
15. Bollinger R, Latif A. Glucose 6 phosphate dehyrogenase deficiency in Lahore area. Pak J Med Res 1985; 24: 85-7.
16. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: A systematic review and meta-analysis. Blood Cells Mol Dis. 2009; 42(3): 267-78. DOI: 10.1016/j.bcmd.2008.12.005.
17. Ronald AR, Underwood BA, Woodward TE. Glucose-6-phosphate dehydrogenase deficiency in Pakistani males. Trans R Soc Trop Med Hyg 1968; 62(4): 531-3. DOI: 10.1016/0035-9203(68)90139-9
18. Khan M. Glucose-6-phosphate dehydrogenase deficiency in adults. JColl Physicians SurgPak 2004;14:400-3.