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Article *in* Journal of Family Medicine and Primary Care · November 2020

DOI: 10.4103/jfmpc.jfmpc_947_20

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Original Article

Assessment of protective relationship of G6PD and other lifestyle factors with Malaria: A case-control study of medical professionals from a teaching medical institute, Gujarat

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Abstract

Background: There remains equivocal evidence in terms of glucose-6-phosphate dehydrogenase (G6PD) and malaria occurrence. A case-control study was performed to assess protective relationship of G6PD and other lifestyle factors with malaria. **Methods:** One-hundred twenty six medical professionals were randomly selected from a tertiary care clinical institute. Along with demographic and lifestyle details, subjects were interviewed about their history of occurrence of malaria at all in previous 10 years. Their hematological, biochemical, and metabolic profile was assessed clinically as well as by investigations. The analysis was carried out with two groups: (1) those who were subjected with malaria at least once in past 10 years (*Malaria Ever Group)*; (2) those who never encountered malaria (*Malaria Never Group).* **Results:** Out of 126, 65 subjects were in *Malaria Ever Group* and 61were in *Malaria Never Group.* There was no difference in lifestyle measures, hematological, and biochemical parameters. Mean G6PD levels were found similar in both the groups. Of 61 subjects in "malaria-never" group, 1 had deficient (1.1 unit/gm of Hb), 9 had low normal (between 2.5 and 10 units/gm of Hb), 48 had normal (10.1–20.5 units/gm of Hb), and 3 had higher than normal (>20.5 units/gm of Hb) G6PD levels. In comparison, 65 participants from "malaria ever" group, none was deficient, 6 had low normal, 58 had normal, and none had higher than normal G6PD levels. HPLC-based hemoglobin analysis showed significant higher number of participants in "malaria-never" group having altered hemoglobin. 12 participants had increased hemoglobin A2 levels, of which 10 were in "Malaria Occurrence Never" group; of them 6 could be diagnosed having hemoglobinopathy of specified variety. 3 of these 10 participants of "malaria-never" group had low G6PD levels also. **Conclusion:** Malaria Protection Hypothesis was not found to be true as per our findings, but there were subtle hints that G6PD protection with or without change in hemoglobin alteration maybe operable.

Keywords: G6PD, hemoglobinopathy, HPLC, malaria ever, malaria never

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Received: 21-05-2020 **Revised:** 31-08-2020
Accepted: 18-09-2020 **Published:** 30-11-2020

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_947_20

Background

Malaria is considered among the global health priorities with 405,000 deaths estimated per year by World Health Organization.[1] Invasion of malaria parasite in normal red

How to cite this article: Pandit N, Kalaria T, Lakhani JD, Jasani J. Assessment of protective relationship of G6PD and other lifestyle factors with Malaria: A case-control study of medical professionals from a teaching medical institute, Gujarat. J Family Med Prim Care 2020;9:5638-45.

blood cell (RBC) leads to acute changes in RBC structure and function with long-term heritable change which has led to survival advantage against malaria.[2] Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme responsible for normal condition of RBCs. The defect in the enzyme causes G6PD deficiency which then leads to hemolysis. Being X-linked hereditary genetic disease, this deficiency is usually prevalent in males than females. There has been presence of several anecdotal evidences, which support protection hypothesis against malaria.[3-5] However, inconsistent definition of G6PD deficiency with mediocre measurement method and lack of evidence-based global consensus on treatment of G6PD-deficient patients demands further research.[6,7]

Indian prevalence of G6PD deficiency as per the meta-analysis conducted by Kumar P. *et al.* was found to be 8.5%.[8] One of the study done recently from Nepal by Sharma U *et al.* reported prevalence of G6PD deficiency of 3% in the population attending tertiary care hospital.[9] In India, few studies carried out on molecular characterization of G6PD with their phenotypic expression in G6PD deficient patients found varied profile of this monogenic disorder.[10] Relation of different mutated G6PD gene and their relation to malaria epidemiology becomes complex because of multiple factors like diversity in climate, ethnicity variance of host, and in local difference of parasite–vector ecology which had resulted in high burden of malaria and resultant deaths in the past.[11] According to WHO world malaria report-2018, 80% of malaria burden in the globe is shared by sub-Saharan Africa and India, of which 50% cases occurred in 5 countries namely Nigeria which accounted for one fourth, Democratic Republic of the Congo for 11%, Mozambique for (5%), and India as well as Uganda for 4% of the cases each.[12] However, India is one of the country where progress is made in eliminating malaria with looking for a way ahead which was acknowledged by WHO, with quoting data of malaria cases of 2017, which was less than the previous year 2016.[12,13]

Malaria epidemiological diversity in India is also reflected to host factors because of tribal population who have G6PD deficiency, hereditary hemoglobinopathy, and other risk factors different from other ancestral groups.[11,14,15] The research conducted so far on G6PD deficiency is limited to only tribal populations and malaria-endemic areas. Thus, we conducted this study in a tertiary care medical institute with medico-professionals and students being subjects of the study.

Malaria is a common disorder for which family physicians are consulted. As recommended by WHO, prioritization in interventions should be tailored to the local epidemiology.[16] Malaria interventions include chemoprophylaxis, lifestyle measures, and safe treatment of malaria occurrence and reoccurrence.[17] Disease burden and transmission can be reduced by preventive treatment strategies and chemoprophylaxis in malaria which is common purview of family physician.[17,18] Primaquine, a drug used as gamatocytocidal for falciparum and hypnozoitocidal for vivax malaria is known to cause hemolytic anemia and other adverse events in G6PD deficient individuals.[19] Health professional engaged in treating as well as preventing malaria at individual or community level, need to know about this very important blood enzyme and its interaction with malaria. Advice by family physicians in regards to life style measures in relation to prevention of infective and non-infective disorders is a common practice. This study on malaria occurrence and reoccurrence is studied for various life styles measures and for common screening investigations ordered for health checkup programmes. Various variables were studied like diet, exercise, yoga, lipid profile, blood sugar, comorbidities, special protective measures, and others; with special reference to G6PD enzyme and abnormal hemoglobin (trait and disease conditions of abnormal hemoglobin).

Methods

After the approval from Sumandeep Vidyapeeth Institutional Ethics Committee, 126 doctors and medical students were consented on random basis from a tertiary care clinical institute and were considered for this study. After informed consent, along with demographic details, subjects were interviewed about their history of occurrence of malaria at all in previous 10 years. They were interrogated for lifestyle details and about their diet. Hematological profile, glucose and lipid profile were done in all the subjects.

The hematological profile included hemoglobin estimation, total and differential WBC count, RBC count, platelet count, packed cell volume (PCV), and blood indices. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), and Red Cell Distribution Width-CV (RDW) were estimated. G6PD measurement was done by UV spectrophotometry. Normal range of G6PD was considered between 10.1 and 20.5 units/gram of hemoglobin, low-normal was considered between 2.75 and 10, deficient when it was below 2.75 and high when it was more than 20.5 units/g of hemoglobin.

The lipid profile included total cholesterol, HDL cholesterol, and triglycerides, whereas VLDL cholesterol and LDL cholesterol were calculated. For glucose profile, FBS/PP2BS/RBS was considered. Mentzer index and Srivastava index were calculated for thalassemia screening and tube turbidity test was done for sickle cell screening.[20,21] Mentzer index was calculated by the mean corpuscular volume (MCV in fL) divided by the RBC count (RBC, in Millions per micro Liter). Mentzer index less than 13 was consider as one of the marker for thalassemia.[20] Similarly, Srivastava index was calculated by dividing MCH by the RBC count. This was also taken as one of the index for differentiation of thalassemia minor from iron deficiency when it was less than 3.8.[21]

Also, HPLC test was carried out as confirmatory test for the respective disease's diagnosis. Zinc protoporphyrin (ZPP) was done on Helena® ProtoFluor Z hematofluorometer (front

face fluorometer). Zinc Protoporphyrin normal range was 20–40 μmol ZnPP/mol Hb.

The analysis was carried out with two groups: (1) Those subjected with malaria ever in past 10 years (Malaria Ever Group); (2) those who never encountered malaria (malaria-never group) at all.

The primary objective for present study was to investigate G6PD-based protection hypothesis in case or malaria. Secondary objectives of this study were to assess differences in hematological indices in subjects with or without encounter to malaria, relationship between anemia and G6PD and alteration in biochemical profile due to encounter to malaria.

Anemia was classified as mild, moderate, or severe based on the concentrations of hemoglobin in the blood. Mild anemia was considered when level of hemoglobin in female was 11–11.9, and in male participants was 11–12 g/dl. It was considered moderate anemia when level was of 8.0–10.9 g/dl, while severe anemia was considered when level was ≤ to 8.0 g/dl.[22]

T-test and Chi-square tests were applied using EPi Info v 7.0 to assess whether difference in distribution was significant in two groups. $P < 0.05$ was considered to be criteria of significance.

Results

Total 126 medico-professionals and students were selected for the study, out of which, there were 84 males (66.66%) and 42 females (33.33%). The mean age of study population was 36.0 ± 12.69 years. There were 86 faculties and 40 students. Out of them, majority population belonged to the Hindu community ($N = 122$; 96.82%). 95 subjects (75.39%) were found to observe pure vegetarianism, whereas 31 subjects (24.60%) were found to have mix type of diet (vegetarian and non-vegetarian). 51.58% of total subjects ($N = 65$) had malaria at least once in their lifetime while 48.41% ($N = 61$) of total subjects did not have a single episode of malaria in lifetime (malaria occurrence never). The baseline characteristics for the both study groups (malaria occurrence never and ever) are given in Table 1.

Mean age of participants of "Malaria Occurrence Never ($N = 61$)" was lower than "Malaria Occurrence Ever ($N = 65$) group, otherwise both groups did not have much differences. Past history of hemoglobinuria or hemolysis was not present in any participant and none were on chemoprophylaxis of malaria. All participants were asked about use of protective measures like mosquito rrepellent measures and use of safety nets There was no significant difference in utilization pattern of these measures between both the groups ($N = 0.1129$) [Table 1]. There was no difference in duration of exercise ($P = 0.9895$) or yoga ($P = 0.2934$) observed in both the groups. Also, the residence in malaria-endemic area was not found to be influential factor ($P = 0.2094$) [Table 1].

Table 2 depicts G6PD profile of subjects in malaria occurrence-"Never" and "Ever" groups. It was found that in both the groups, mean G6PD levels were found similar regardless of occurrence of malaria ($P = 0.8093$). When levels of G6PD were compared in total 126 participants, they were in normal range (*Normal, >10 in male and female)* in 48 out of 61 (78.69%) in "malaria-never" and in 58 out of 65 (89.23%) in "malaria-ever" group. G6PD deficient (G6PD less than 2.75 units/g of hemoglobin) was present in one male subject; a participant from "malaria-never" group. Low normal level (between 2.75 and 10 units/g of hemoglobin) of G6PD ("Not deficient" but "lower than normal G-6PD") was present in 16 participants (13.59%). Of these 16 participants 09 were in "malaria-never" and 07 were in "malaria-ever" group. Participants having such levels can be heterozygotes female or hemizygote male having low levels.[23,24] Of total 17 (16 having low normal and 1 participant of deficient), 13 were male and 4 females. Interestingly, higher than normal (>20.5 units/gram of hemoglobin) was present in 3 participants; 2 males and 1 female, all of them in "malaria-never" group. Thus, levels which can be considered to be normal for G-6PD were more in "malaria-ever" group than "malaria-never" group. (78.69% normal in malaria-never and 89.23% in malaria-ever group). [Table 2]

Comparative analysis of blood cell counts (total WBC, RBC, and platelet count) in both groups showed no significant difference. The mean hemoglobin levels were similar in both the groups with no significant difference ($P = 0.9655$). Category wise comparative analysis for PCV, MCV, MCH, MCHC, and RDW in both groups were similar in terms of blood cell morphology except in HPLC report which showed hemoglobin A2 > 4.0 in 12 subjects of which in 10 were of malaria-never group. This result was statistically significant. [Table 3]

There was no difference in terms of malaria history distribution between anemic and non-anemic population. The mean Hb levels were similar in both the groups with no significant difference ($P = 0.9655$).

Table 4 represents sub-analysis of 12 participants of which first 10 listed were in "Malaria Occurrence Never" group, while rest 2 were in "Malaria Occurrence ever" group. Participants listed in Table 4, numbered as no 2, 4, 6, 8, 11, and 12 had increased hemoglobin A2 levels and were advised further workup. Others could be diagnosed having hemoglobinopathy of specified varieties.

Discussion

A cytosolic enzyme G6PD protects RBCs from reactive oxygen species and its prime function is processing of carbohydrates. If mutations in the G6PD gene occur, it may reduce the amount of glucose-6-phosphate dehydrogenase. It is believed that subjects having G6PD mutation may be partially protected against malaria as parasite finds difficulty in invading RBCs. Present study was performed to find out relation of G-6PD levels and other host factors in relation to malaria.

Table 1: Baseline Characteristics; (*n*=126)

	Malaria Occurrence Never (*n*=61)	Malaria Occurrence Ever (*n*=65)	*P*
Age (Years)	34.18±11.32	38.70±13.55	0.0485
Gender			
Male	39 (30.95%)	45 (35.71%)	0.2679
Female	22 (17.46%)	20 (15.87%)	
Religion			
Hindu	58 (46.03%)	64 (50.79%)	0.2435
Others	3 (2.38%)	1 (0.79%)	
Height (cm)	167.01±8.27	166.82±8.84	0.8996
Weight (kg)	66.76±14.81	67.98±11.54	0.6071
BMI	23.62±5.76	24.36±3.50	0.3856
Pulse	78.35±8.65	77.23±6.89	
Blood Pressure			
SBP	116.05±11.98	120.90±13.20	0.0958
DBP	75.24±6.88	73.35±12.91)	0.4295
Diet			
Vegetarian Only	40 (31.74%)	56 (44.44%)	**0.0031**
Non-Veg+ Veg	21 (16.66%)	9 (7.14%)	
Exercise/Yoga			
Total Minutes per Week (E)	85.36±167.06	85.79±132.41	0.9895
Total Minutes per Week (Y)	10.60±34.68	24.54±77.44	0.2934
Protective Measure			
Regularly	28 (22.22%)	18 (14.28%)	0.1129
Seasonally	22 (17.46%)	28 (22.22%)	
Occasionally	9 (7.14%)	15 (11.90%)	
Nil	2 (1.58%)	4 (3.17%)	
Residence in Endemic Area			
Yes	38 (30.18%)	46 (36.50%)	0.2091
No	21 (16.66%)	15 (11.90%)	
No Data	2 (1.58%)	4 (3.17%)	
FBS >100			
Yes	15 (11.90%)	14 (11.11%)	0.3452
No	46 (36.50%)	51 (40.47%)	
Haemoglobin g/dl	13.52±1.71	13.54±1.82	0.97
Co-morbidity*			
Yes	7 (5.55%)	11 (8.73%)	
No	54 (42.85%)	54 (42.85%)	
Lipid Profile (Mean+-SD)			
LDL	102.60±30.37	104.87±30.95	0.3564
HDL	39.49±7.71	39.31±7.55	0.8962
VLDL	30.33±16.67	29.71±16.38	0.8351
Tri-Glycerides	152.66±+-82.56	140.18±73.07	0.3819
Cholesterol	172.36±+-39.17	171.86±32.37	0.9390
SGPT	32.22±24.53	24.46±10.46	**0.0277**
S. Creatinine	1.073±0.14	1.077±0.14	0.8634
Glucose Profile (Mean+-SD)			
FBS	94.07±24.60	96.55±21.11	0.5553

*Co-morbidities observed in present study were ischemic heart disease, diabetes, hypertension, hypothyroidism etc

This study was performed in a teaching medical institute and the study subjects were medico-professionals and students of medical college. The reason behind selection of such subjects and settings was that as the study aimed to assess history of malaria encounter in past 10 years, medical professionals could provide comparably accurate response. Moreover, result influence due to factors like forgetfulness, non-compliance to healthy lifestyle measures in such subjects is of lesser concern. Secondly, this study assessed long term effects of malaria on hematological as well as biochemical profile of the subject with focused attention to G6PD,which was first in its kind,different than studies referred in a review article by Marrelli M T *et al*; which assessed the long term changes in cardiac and skeletal muscles due to malaria and anti malarial drugs.[25] The male-female ratio in our study was 2:1, which was considered to be good support in assessing G6PD deficiency's gender specificity evidenced in published studies.

Table 2: G6PD levels

G6PD levels in units/gram of haemoglobin	Malaria Never (*n*=61) (M=39, F=22)	Malaria Ever (*n*=65) (M=45, F=20)	*P*
Mean Level of G6PD (*n*=126)	12.01±3.45	12.13±2.28	0.81
Mean Level of G6PD in male (*n*=84)	11.8±2.96 (Range between 1.1 to 25.3 in 39 males)	11.5±2.82 (Range between 6.4 to 17.7 in 45 males)	0.63
Mean Level of G6PD in Female (*n*=42)	12.3±2.89 (Range between 6.4 to 21.0 in 22 females)	13.7±2.80 (Range between 9.5 to 18.4 in 20 females)	0.11
Deficient <2.75)	1 (0.79%) (male subject having G6PD of 1.1 units/gram of Hb)	0 (0%)	
Lower than Normal, (>2.75 and <10)	09 (14.75%) (06 males and 3 females)	07 (10.77%) (06 males and 01 female)	0.5
Normal, >10 (male and female)	48 (78.69%)	58 (89.23%)	0.17
Higher than Normal (>20.5)	3 (2 Males and 1 female)	0 (0%)	
Abnormal G6PD level in male (*n*=84)	09 (23.07%) (10.71%)	06 (13.33%) (7.14%)	0.27
Abnormal G6PD level in female (*n*=42)	04 (18.18%) (9.52%)	01 (5%) (2.38%)	0.35

Table 3: Hematological parameters

	Malaria never (*n*=61)	Malaria ever (*n*=65)
Mean Hb Level	13.52±1.71	13.54±1.82
Mild Anaemia	7 (5.55%)	6 (4.76%)
Moderate Anaemia	5 (3.96%)	5 (3.96%)
Severe Anaemia	0 (0%)	1 (0.79%)
No Anaemia	49 (38.88%)	53 (42.06%)
Zinc Protoporphyrin in µmol ZnPP/mol Hb	24.24±11.69	28.50±22.98
MCV in fl	85.5±8.9	84.4±7.9
MCH	28.0±3.3	27.5±3.3
MCHC	32.7±4.0	32.6±3.1
PCV	41.3±4.8	41.5±4.8
RDW-CV	13.67±1.61	13.83±1.61
RDW*MCV	1169±121	1164±121
Mentzer index	17.3±3.0	18±3.1
Srivastava index	5.7±1.2	5.8±1.2
Mentzer index <13	4	3
Srivastava index <3.8	4	3
Haemoglobin A2 >4.0	10	2 (*P*=0.0141) (statistically significant)

Mukherjee M.B *et al.* in review paper describes Indian scenario of G6PD deficiency among tribal populations of India.[26] In India, work is more into certain race and tribe and to thus we intended in this to do screening in medical professionals and to link other host factors as cause or effect to malaria.

The study results showed that there was no difference in G6PD levels for subject with and without malaria encounter once in their lifetime. As per definition of relative G6PD deficiency, present study found 17 participants (13.59%) relatively G6PD deficient. This prevalence was similar to that reported in a Srilankan study by Gunawardena *et al.* which was 13.95%.[27]

Though our result did not show significant association between G6PD levels and malaria encounter during lifespan, one male participant who was having G6PD deficieny had level of 1.1 units/g of hemoglobin and was in "malaria occurrence-never" group. Severe form of G6PD dehydrogenase deficiency occurs in males, being X-linked. G6PD deficiency may be cause of hemolysis and may result in anemia, indirect bilirubnemia, and clinical jaundice especially exposed to infections. Participant who had severe G6PD deficiency did not have any symptoms and was unaware of the G6PD status.

Comparative less numbers of participants of "malaria-never group" had normal range of G6PD in comparision to in "malaria-ever group" (78.69% vs. 89.23%, respectively). Few hints from this study suggests possibilty in favor of protective hypothesis, however due to less power of the study, significant difference was not derived. One interesting fact which had emerged was that 3 participants (2 male and one female) in "malaria-never group" had higher than normal G6PD levels. Higher level canbe due to polymorphic nature of gene transmission and it may be possible that it also may offer protection from to malaria as it was present only in malaria-never group. Increased G6PD expression in adipocytes is reported due to oxidative stress and NF-κB signaling, related to obesity and metabolic syndrome.[28] All these three subjects did not have features of metabolic disorder.

G6PD deficiency is reported from areas known to be affected by malaria. It is common in Africa, Asia, and Mediterranean countries. In India, the prevalence varies from 2.3 to 27.0% with an overall prevalence of 7.7% in different tribal groups.[26] Study in Southern Pakistan was done to evaluate the frequency of G6PD Mediterranean in male individuals with and without falciparum malaria.[29] G6PD-Mediterranean is one variant characterized by severe enzyme deficiency and B-like electrophoretic mobility.[30] Though frequency for this G6PD allele was different in malaria-patients in comparison with healthy individuals they concluded that there is a need of large studies including females to find out true burden of G6PD in malaria-endemic areas.[29]

Is there a need of G6PD screening testing and also of G6PD research in relation to malaria? G6PD screening is recommended in tribal populations of India who lives in malaria endemic area where anti- malarial drugs can result in hemolysis which in turn can lead to morbidity and mortality.[26] However, research is also necessary in relation to G6PD and occurrence of malaria in non-tribal population. Our paper deals with the screening of G6PD in heterogeneous group of participants' especially medical

Table 4: Hematological parameters & Sub analysis of 12 participants who had Hemoglobin A2 >4.0

N/E	G	Age	Hb in gm%	MCV fl	MCH pg	MCH C%	RBC Mil/ µl	RDW -CV %	G-6-PD unit/g of Hb	ZPP µmol ZnPP/ mol Hb	Mentzer index	Srivastava index)	HPLC comments
N	M	26	13.2	65.8	20.3	30.8	6.5	18.6	18.3	59	10.1	3.1	A2=5.5%, BETA THALASSEMIA MINOR
N	M	30	15.6	90.4	29.3	32.4	5.32	14.6	8.8	38	17.0	5.5	HBA2=5.5%, Further study
N	F	28	9.6	63.9	19.3	30.2	4.98	15.6	11.7	26	12.8	3.9	HBA2=5.6%, BETA THALASSEMIA TRAIT
N	M	28	14.9	86.1	28.7	33.3	5.19	14	9.2	14	16.6	5.5	HBA2=4.6%, Further study
N	F	25	13.3	84.7	27.5	32.5	4.83	13.1	10.9	26	17.5	5.7	HbD IRAN TRAIT
N	M	28	15.9	94.1	32.1	34.1	4.95	14	13.1	21	19.0	6.5	HBA2=4.4%, Further study
N	F	27	12.9	89.0	30.7	34.5	4.2	13.5	10.0	32	21.2	7.3	HbD PUNJAB TRAIT (FLASELY ELEVATED HBA2)
N	M	25	15.8	85.6	27.4	32.0	5.74	14.5	12.2	12	14.9	4.8	HBA2=4.4%, Further study
N	M	36	13	63.9	19.4	30.3	6.71	17.3	21.0	67	9.5	2.9	HBA2=5.8%, BETA-THALASSEMIA MINOR
N	M	31	14.6	80.8	27.2	33.6	5.37	12.1	11.6	30	15.0	5.1	HBA2=5.3%, BETA-THALASSEMIA MINOR
E	F	25	12.7	85.2	27.1	31.8	4.67	12.9	10.8	22	18.2	5.8	HBA2=4.4%, DISTORTED PEAK
E	M	25	13.1	80.6	25.1	31.1	5.23	15.8	11.0	26	15.4	4.8	HBA2=4.5%, Further study

professionals. Our study group had some participants from tribal area also who had come to our center for employment or had come for further studies from all over India. We studied their metabolic and biochemical profile which was similar in "Malaria Occurrence Never" and "Ever" groups. Literature in regards to G6PD do mention about these areas of scientific lacunae.

Intermediate G6PD activity (30–60%), such as heterozygous females is always an interesting area of research.[31] In our study data, 9 (14.75%) (6 males and 3 females in "Malaria Occurrence Never" group and 07 (10.77%) (06 males and 01 female) in "Ever" group had intermediate G6PD activity, (>2.75 and < than 10 units/g of hemoglobin). From Gujarat, a study was done in pairs of spouses from Vatalia Prajapati community, known for high prevalence of G6PD deficiency. Classification of enzyme activities (as EU/g Hb at 37°C) was done as "Normal" when level was > 9 (male and female); Heterozygote, 2.75 – 8.99 (female), and Deficient < 2.75 (male hemizygote and female homozygote). They studied 148 subjects of which 40 of 77 male and 25 of 71 female were deficient for G6PD enzyme. There was difference in blood indices and hematological parameters in normal G6PD and deficient G6PD subjects.[23,32]

Study by Kotepui, M. *et al.* on malaria parasite density on blood cell parameters had found significant changes in most of the blood cell parameters.[33] Long-term impact of malaria and G6PD deficiency on hematological profile includes anemia especially in pregnant women in tropical countries is reported by Engwa *et al.*[34] We studied hematological parameters also in both our groups. Though we did not find significant difference, hemoglobin analysis by HPLC showed 12 participants who had increased hemoglobin A2 levels, of which 10 were in "Malaria Occurrence Never" group, while rest 2 were in "Malaria Occurrence ever" group. Of them 6 could be diagnosed having hemoglobinopathy of specified varieties. Interestingly of this 12, three were having low level of G6PD. Statistically significant number had hemoglobinopathy in "malaria-never" group than the other group. Findings like this may suggest that there are heritable change in RBC enzyme (one of important being G6PD) associated with change in hemoglobin structure which gives protection from malaria. Sub analysis of these 12 participants revealed that 4 participants had beta thalessemia trait, one had HbD Punjab trait, and one had HbD Iran trait all from "malaria occurrence never" group. Heterozygous form of the HbD does not lead to any symptoms. Our area, Gujarat, Western part of India have Sickle hemoglobinopathy and its variant common including Hemoglobin D-Punjab (also known as Hb D- Los Angeles).[35]

There are various host factors linked to malaria occurrence and their severity, like ABO blood group, hemoglobinopathies, and G6PD deficiency linked to malaria, all of which common in malaria endemic zones of past and present.[36] Literature in this regards are varying and is of divergent opinions. Our study gives subtle hint that changes in RBC enzymes and hemoglobin variation may have role in malaria protection, however due to changing epidemiology of present day clear results from the available literature is not emerging. Apart from change in malaria epidemiology, environmental change, change in herd-immunity, inter-caste/inter-race marriages and due to migrating population these humane genetic host factors are not reflected very clearly as protective or risk factors. Nevertheless, treating doctors all over the globe has to understand these protective/risking factors for better management of malaria and for interventional policy making; locally as well as globally which can be tailored to local contexts.[15,17]

This study shows that G6PD levels in association with hemoglobin alteration have some protective role in malaria prevention. This study also examined other life style factors like diet, exercise, yoga, lipid profile, blood sugar, comorbidities, special protective measures, which was similar in both groups in malaria ever and never groups except in diet where significant difference was observed in favor of mixed diet (Vegetarian + Non-vegetarian) in malaria never group. This study also shows that medical professional screening in relation to protecting and risking factors in malaria could be varied and important and there is equal need

of access to care to medical fraternities along with conventionally chosen population for malaria related community intervention. It was also found that there was utmost need to provide awareness about protective measures as they were, regardless of their types and designs, were found under-utilized.

There were several limitations of this study: (1) the study population reported verbally about their history of "malaria-ever "in previous 10 years. This may be considered as source of recall bias; (2) secondly, the gene responsible for G6PD deficiency has several variants, which were not considered under scope of this study. Moreover, type of malaria (i.e., P. Falciparum or P. Vivax) was also not studied due to methodological constraints; (3) lastly, the sample size of this study, being exploratory research was small. Broad-base screening of general population in relation to G6PD, hemoglobinopathy, and malaria occurrence may be needed for more clear conclusions which can give insight to many other infections also.

Conclusion

Malaria protection hypothesis was not found to be true as per our findings, but there were subtle hints that G6PD protection hypothesis maybe operable and further similar research in general community may give clear idea. Policy focus only in malaria endemic areas and specific population which is though required general susceptibility, host criteria including research in hemoglobinopathy with or without G6PD levels in malaria is needed.

Acknowledgements

Our first and fore most thanks are to study participants who kindly consented and gave inputs in this project. We thank the authorities of Sumandeep Vidyapeeth deemed to be University, Pipalia, and Dist: Vadodara for granting funds for this project. We also thank Dean Dr. G. V. Shah, SBKS Medical Institute institution and Research Institute and Dr. A.K. Seth, Research Director, Sumandeep Vidyapeeth deemed University who allowed us to conduct this study. We are also thankful to Medical Superintendent, Dhiraj Hospital, Heads of Constituent colleges and Heads of departments of our institute and University for helping us for successful completion of this study. We are thankful to Mr. Vatsal Chhaya, Research officer for his help in statistical analysis of this study project.

Ethics declarations

Ethics approval and consent to participate: This study was approved by Sumandeep Vidyapeeth Ethics Committee. Each participant signed, informed consent.

Financial support and sponsorship

The study is financially supported by Sumandeep Vidyapeeth Research Fund.

Conflicts of interest

There are no conflicts of interest.

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