International Journal of Hematological Disorders, 2014, Vol. 1, No. 1, 31-34
Available online at http://pubs.sciepub.com/ijdh/1/1/6
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DOI:10.12691/ijhd-1-1-6

Effect of G_6pd Status in Malaria Infected Individuals on Cortisol, Malondialdehyde, Blood Glucose and Blood Lipid Profile

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Received October 31, 2014; Revised November 08, 2014; Accepted November 10, 2014

Abstract The aim of this study was to determine the effect of Glucose-6-Phosphate dehydrogenase (G6PD) status in Plasmodium falciparum malaria infected individuals cortisol, malondialdehyde (MDA), blood glucose and blood lipid profile. The study was conducted at Federal Medical Centre, Ido-Ekiti, Nigeria. Two hundred and two blood samples were collected twice from each malaria infected individuals. Thick blood film was made and stained with Giemsa's staining technique for malaria parasite detection; Glucose-6-Phosphate dehydrogenase (G-6-PD) was performed using methaemoglobin reduction method. Data obtained was analysed using SPSS version 16. The result of this present study showed that the mean±SD of cortisol and malondialdehyde (MDA) in G6PD normal were higher compared to G6PD deficient in pre and post anti-malaria drug treatment. Cortisol levels increased in patients with P.falciparum malaria infection, which decline as the clinical condition improved and parasitaemia decrease during anti malaria treatment.

Keywords: *malaria parasite, G_6PD and anti-malaria therapy*

Cite This Article: Esan A.J, and Ifeanyichukwu M.O, "Effect of G_6pd Status in Malaria Infected Individuals on Cortisol, Malondialdehyde, Blood Glucose and Blood Lipid Profile." *International Journal of Hematological Disorders*, vol. 1, no. 1 (2014): 31-34. doi: 10.12691/ijhd-1-1-6.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) plays a crucial role in preventing oxidative damage to human red cells by participating in the generation of antioxidants. G6PD deficiency is due to diminished activity of the enzyme glucose-6-phosphate dehydrogenase which catalyses the first and rate controlling step in the hexose monophosphate shunt metabolic pathway [1]. G6PD-deficient individuals show the symptoms in response to one or more oxidative stresses. Red blood cells are lived for 120 days, it is highly specialized cells which function as oxygen and carbon dioxide transporter and lack most of the organelles including the nucleus. The role of red cells as oxygen carriers puts them at substantial risk of damage from oxidizing free radicals except for the protective effect of G6PD/NADPH/glutathione. The G6PD/NADPH pathway is the *only* source of reduced glutathione in red blood cells, thus lack of G6PD enzyme in the red blood cells is lethal and deficiency in the enzyme in case of oxidative stress is deleterious to the cell. Any oxidative stress in the red blood cells with deficient G6PD enzyme may result in haemolytic anaemia. People with G6PD deficiency are therefore at risk of haemolytic anaemia in states of oxidative stress. G6PD deficiency causes increased susceptibility of erythrocytes to H_2O_2 and other reactive oxygen species that can lead to haemolytic anaemia. Oxidative stress can result from infection, chemical exposure to medication and certain foods. Malaria parasites break down haemoglobin after invasion. They do so to make room to grow and may also derive nutrition from it. The by-product of this process, particularly the oxidized iron is potentially toxic to the parasite. Reduced glutathione (G-SH) supplies reducing energy to cells and is the natural mechanism of cells to overcome the oxidative stress. Any deficiency in the production of G-SH in the cell can provide resistance against the malaria parasite. Reduced glutathione functions as an antioxidant and protects the cells against oxidative stress by mop up free radicals that cause oxidative damage. Since malaria is a disease with high morbidity and high mortality, it therefore has a powerful selective force in human populations, the maintenance of a high frequency of G6PD deficiency despite its deleterious effect of haemolysis is due to its property as a defence against malaria [2,3]. The aim of this study was to determine the effect of G6PD status in *Plasmodium falciparum* malaria infected individuals on cortisol, malondialdehyde (MDA), blood glucose and blood lipid profile.

2. Materials and Methods

2.1. Subjects and Study Design

This study was conducted at Federal Medical Centre, Ido-Ekiti, Nigeria. Subjects were *Plasmodium falciparum* malaria infected adult individuals; presented with signs and symptoms of malaria infection. This was confirmed using malaria rapid kit test and microscopy detection of malaria parasite. Two hundred and two blood samples were collected (10 ml) twice from each malaria infected individuals; grouped as pre-treatment (at presentation) and post anti-malaria drug treatment. One hundred and two blood samples from apparently healthy individuals negative to malaria infection was collected as control; both *Plasmodium falciparum* malaria infected subjects and controls were within the age 15-64 years of both sex. Patient's consent was sort for through an informed consent form; also ethical approval was obtained from the hospital. Structured questionnaire was used to obtained demographic characteristic and other relevant information for the study.

2.2. Sample Collection

Ten millimetres (10 ml) of blood sample was collected from each subject on the first day of visiting hospital as baseline sample after the patient has been clinically diagnosis for malaria infection, another 10 ml of blood sample was collected on the second or third day after taking anti-malaria drugs. 3 ml of blood sample was dispensed into plain bottles; serum was extracted to assay stress index hormone (cortisol) and malondialdehyde (MDA), 1ml of blood sample was dispensed into fluoride oxalate bottles to assay blood glucose level, 3 ml of blood sample was collected into lithum heparin bottle to assay lipid profile and 3 ml of blood sample was dispensed into di-potassium ethylenediaminetetracetic acid (K_2EDTA) vaccutainer bottles for malaria parasite detection on thick blood film and for determination of G6PD status of the participants.

2.3. Methodology

Thick blood film was made and stained with Giemsa's staining technique for malaria parasite detection; observed under microscopy using x100 objective lenses, the procedure was described by Monica Cheesbrough [4]. Glucose-6-Phosphate dehydrogenase (G-6-PD) was performed using methaemoglobin reduction method within 6 hours of sample collection; the procedure was as described by Dacie and Lewis [5]. Cortisol was estimated using enzyme linked immunosorbent assay (ELISA) method by Monobind Inc. Lake Forest, CA 92630, USA; the procedure was as described by the manufacturer of the kit. Malondialdehyde (MDA) was estimated using thiobarbituric acid method by Tomotsu. Briefly; 0.5 plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. 1 ml of 0.6% 2-thiobarbituric acid (TBA) was added to the mixture, shaken, and warmed for 30 min in a boiling water bath followed by rapid cooling. Then it was shaken into a 4 ml of nbutyl- alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 535 and 520 nm by spectrophotometer against butanol. The standards of 5, 10, 20 nmol/ml of 1,1,3,3-tetraethoxypropane (TEP) were used. The results were expressed as nmol/ml plasma [6] However, blood glucose was estimated using glucose oxidase method, the procedure was as described by the manufacturer of the kit (Randox); 0.01 ml of blood sample was pipette into a labelled clean test tube, 0.01 ml of reagent standard was pipette into another labelled clean test tube, 1.00 ml of glucose reagent was pipette into each of the test tube, water blank was used; contents in the test tube was mixed and incubated for 10 minutes at 37°C. Absorbance was read at 500 nm. Lipid profile majorly consist of high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides and cholesterol. Lipid profile was estimated using CHOD-PAP method; the procedures were as described by the manufacturer of the kit (randox); Low density lipoprotein was calculated by Freidewald formular.

3. Statistical Analysis

Data obtained were analysed for mean and standard deviation; significant test was done by student t- test. Level of significance was considered as <0.05.

4. Result

Table 1: show mean ± SD of biochemical parameters on G6PD status in pre, post anti-malaria drug treatment and control. Biochemical parameters include cortisol (µg/dL), MDA (nmol/L), glucose (mmol/L) HDL (mg/dL), LDL (mg/dL), Triglyceride (mg/dL) and total cholesterol (mg/dL). The mean ± SD of cortisol 22.65 ± 3.98 in pre treatment G6PD normal was significantly (P<0.05) higher compared to 22.30 ± 2.77 in pre treatment G6PD deficient also mean ± SD of MDA 20.19 ± 3.06 in pre treatment G6PD normal was higher compared to 18.29 ± 2.31 in pre treatment G6PD deficient; comparison show no significant difference (P>0.05). However, the mean ± SD of glucose, HDL LDL, Triglycerides and total cholesterol 4.96 ± 0.73, 29.16 ± 4.52, 52.78 ± 15.53, 77.51 ± 19.99 and 97.42 ± 15.39 respectively in pre treatment G6PD normal were lower compared to. 54 ± 0.70, 30.02 ± 5.22, 54.59 ± 17.86, 79.72 ± 20.54 and 100.56 ± 16.98 in pre treatment G6PD deficient. The comparison show no significant difference (P> 0.05); the mean ± SD of cortisol and glucose 16.39 ± 7.25 an 4.33 ± respectively in post treatment G6PD normal were significantly (P<0.05) lower compared to mean ± SD of cortisol and glucose 17.72 ± 3.69 and 4.48 ± 0.67 respectively in post treatment G6PD deficient; also, mean SD of MDA 15.85 ± 6.18 in post treatment G6PD normal was significantly (P<0.05) higher compared to MDA 13.83 ± 3.28 in post treatment G6PD deficient. However, mean ± SD of HDL, LDL, Triglycerides and total cholesterol 27.42 ± 3.41, 57.99 ± 11.63, 86.50 ± 17.36 and 102. 67 ± 11.84 respectively in post treatment G6PD normal were lower compared to 27.59 ± 3.54, 60.11 ± 13.67, 87.94 ± 17.36 and 102.67 ± 11.84 respectively in post treatment G6PD deficient; comparisons show no significant difference (P>0.05). Furthermore, the mean ± SD of cortisol, MDA and glucose 6.59 ± 1.59, 8.25 ± 0.87 and 3.57 ± 0.31 respectively in control G6PD normal were significantly (P<0.05) lower compared to 6.62 ± 1.90, 8.90 ± 0.67 and 3.86± 0.30 in control G6PD deficient also mean ± SD of

HDL, LDL, Triglyceride and total cholesterol 42.09 ± 3.61, 62.87 ± 6.87, 97.05 ± 11.68 and 124.35 ± 10.32 respectively in control G6PD normal were higher compared to mean ± SD of HDL, LDL, Triglyceride and total cholesterol 41.91 ± 4.30, 60.64 ± 7.09, 93.73 ± 11.36 and 121,36 ± 10.09 respectively in control G6PD deficient. The comparison show no significant difference (P>0.05).

Table 1. Means ± sd of biochemical parameters in pre on G_6pd status in pre treatment, post-antimalaria drug treatment in malaria infected subjects and control

Groups	Pre Treatment			Post Treatment			Control		
Parameters	Normal (N=148)	Deficient (N=54)	P value	Normal (N=148)	Deficient (N=54)	p. value	Normal (N=91)	Deficient (N=11)	p. value
CORTISOL µg/dL	22.65±3.98	22.30±2.77	0.00*	16.39±7.25	17.72±3.69	0.00*	6.59±1.59	6.62±1.96	0.45
MDA nmol/L	20.19±3.06	18.29±2.31	0.43	15.85±6.18	13.83±3.28	0.00*	8.25±0.87	8.90±0.67	0.45
GLUCOSE mmol/L	4.96±0.73	5.54±0.70	0.18	4.33±0.95	4.48±0.61	0.00*	3.57±0.31	3.86±0.30	0.66
HDL mg/dL	29.16±4.52	30.02±5.22	0.11	27.42±3.41	27.59±3.54	0.62	42.09±3.61	41.91±4.30	0.56
LDL mg/dL	52.78±15.53	54.59±17.86	0.15	57.99±11.63	60.11±13.67	0.14	62.87±6.87	60.64±7.09	0.95
TRIG mg/dL	77.51±19.99	79.72±20.54	0.67	86.50±17.36	87.94±18.38	0.51	97.05±11.68	93.73±11.36	0.48
TOTAL CHO mg/dl	97.42±15.39	100.56±16.98	0.39	102.67±11.84	105.30±13.46	0.26	124.35±10.32	121.36±10.09	0.19

P<0.05 Significance, P>0.05 no Significant

5. Discussion

Out of 202 *P. falciparum* malaria patients used in this study, 148 (73.3%) were G6PD normal and 54 (26.7%) were G6PD deficient, among the control group, 91 (89.2%) were G6PD normal and 11 (10.8%) were G6PD deficient. Prevalence of G6PD status in this present study was similar to Francis *et al*., [7] reported of the four (400) individuals screened for this G6PD deficiency, 347 (86.75%) had normal G6PD levels and 53 (13.25%) were G6PD deficient of which 36 (9.0%) were heterozygous and 17 (4.25%) were homozygous. The high frequency of G6PD deficiency in the study population corroborates the role malaria play in the distribution of G6PD genes in most malaria endemic areas in the world [8]. G6PD status in patient infected with *plasmodium falciparum* showed that the mean value of cortisol and MDA were observed higher in G6PD normal compared to G6PD deficient, this study showed the extent of stress induced by malaria parasite infection and oxidation stress produced in malaria infection in pre-treatment; However, the level of cortisol and MDA were significantly decrease in post treatment. Although, the mean value in G6PD normal was slightly lower compared to G6PD deficient; this was due to the effect of anti-malaria drug used during malaria treatment. Moreover, mean value of cortisol and MDA in control were significantly lower compared to pre treatment which showed the extent of stress induced by malaria infection and oxidative stress produced by malaria infection also, while comparing control values with post treatment showed greater extent of malaria recovery after treatment with evidence of reduced stress index (cortisol) and decrease in oxidative stress (MDA) produced by malaria parasite. Mean value of glucose in G6PD deficient was observed higher compared to G6PD normal in pre treatment, post treatment and control; the slight increase in glucose level of G6PD deficient was corresponded to the extent of stress induced by malaria parasite; measured by cortisol. Similar to this present study, Francis and Pete [9] stated that hypoglycaemic was found to be more common in G6PD non deficient subjects compared to G6PD deficient. Hypoglycaemia was evident at malaria presentation and could be due to impaired hepatic gluconeogenesis and increased consumption of glucose in peripheral tissue as well as by parasite. Gluconeogenesis probably failed to compensate, in the presence of decreased glycogen flux of glucose, increasing the risk of hypoglycaemia in *falciparum* infected subjects as reported by Dekker *et al*., [10]. However, in post treatment, blood glucose was significantly decrease in G6PD normal and G6PD deficient this showed treatment improvement while companying post treatment value to control. Mean value of lipid profile (HDL, LDL, Triglycerides and total cholesterol) in G6PD normal were observed lower compared to G6PD deficient in pre treatment and post treatment. This study support the fact that G6PD deficient had genetic resistance to malaria infection and also result of lipid profile in this study showed the extent of stress induced by malaria parasite. However, in post treatment, there was improvement in the lipid profile level due to anti malaria drug used during treatment. Lipid profile in control was within the normal range, although there is no statistical significant difference in the parameters. Comparing the mean values of lipid profile in pre treatment and control showed the extent of stress induced by malaria infection; while comparing the mean values in post treatment to control, this study showed the extent of improvement due to anti malaria drug used.

6. Conclusion

This present study showed that cortisol levels increased in patients with *P. falciparum* malaria infection which decline as the clinical condition improved and parasitaemia decrease during anti malaria treatment. During malaria infection, cortisol stimulates gluconeogenesis which increased plasma glucose concentration and decreased during malaria treatments.

References

[1] Luzzatto, L., Mehta, A. & Vulliamy, T. In: C.R. Scriver, A.L. Beaudet, W.S. Sly & D. Valle (eds) The Metabolic and Molecular Bases of Inherited Diseases. McGraw-Hill, New York, (2001) pp 4517-4553.

[2] Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood;* (2008) 111: 16-24.

[3] Luzzatto L, Usanga EA, Reddy S. Glucose-6-Phosphate Dehydrogenase Deficient red cells: Resistance to infection by malarial parasites. *Science;* (1969) 164: 839-842.

[4] Monica Cheesbrough. Discrete Laboratory Practice in Tropical Countries Part 1, Cambridge Second Editions. Published by Press Syndicate of the University of Cambridge, (2005) chp. 5, page 247-258.

[5] Dacie and Lewis. Pratical Haematology 10th ed. Churchill Livingstone Elsevier (2006) chap. 10: 219-220

[6] Nurten Tüközkan, Hüsamettin Erdamar, Ilgım Seven. Measurement of Total Malondialdehyde in Plasma and Tissues by High-Performance Liquid Chromatography and Thiobarbituric Acid Assay *Fırat Tıp Dergisi;* (2006)11 (2): 88-92.

[7] Francis M. Awah, Nwanedo Chukwuemeka G., Salami Ibrahim Olalekan, Augusta Ehijie Azeke, Mbaike Nneka. A possible protective role of glucose-6-phosphate dehydrogenase deficiency and sickle haemoglobin genes against severe malaria in Madonna University, Elele Community *Journal of Medicine and Medical Sciences* (2012) Vol. 3 (6) pp. 375-381

[8] El-Hazmi MAF, Warsy AS. The Frequency of Glucose-6-Phosphate Dehydrogenase Phenotypes and Sickle Cell Genes in Al-Qatif Oasis. *Annals of Saudi Med*. (1994) 14 (6): 491-494.

[9] Francis M. AWAH and Pete N. UZOEGWU. Influence of sickle heterozygous status and glucose-6-phosphate dehydrogenase deficiency on the clinico-haematolgoical profile of *Plasmodium falciparum*-infected children *Biokemistri* (2006) 18 (2): 89-97.

[10] Dekker E, Romijn JA, Ekberg K, Wahren J, Van Thien H, Ackermans MT, *et al*., Glucose production and gluconeogenesis in adults with uncomplicated falciparum malaria. *Am J Physiol;* (1997) 272. E1059-*1064.*