Indian Journal of Clinical Biochemistry, 2009 / 24 (3) 288-293

EVALUATION OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS OF PREGNANT WOMEN SUFFERING FROM MALARIA IN CAMEROON

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ABSTRACT

Oxidative stress is thought to be involved in the pathophysiology of malaria, especially in pregnancy where natural resistance is markedly reduced. In the present study we investigated oxidative stress in 315 pregnant women out of which 159 had Plasmodium falciparum malaria and 154 controls. We evaluated the level of lipid peroxidation products (MDA level) in the plasma, the activity of erythrocyte antioxidant defense enzymes, superoxide dismutase (SOD, EC: 1.15.1.1) and catalase (Cat, EC: 1.11.1.6) as well as the ability to resist oxidative stress by the FRAP (Ferric Reducing Ability of Plasma) assay. Total erythrocyte protein levels were also examined. For the two groups of patients, several differences between the biochemical parameters tested were found. Median parasitaemia in women with malaria was 25,392 parasites/µl of blood (Range1200 - 82000), while in controls we had no parasites found in thin and thick smears. Levels of lipid peroxidation products (MDA) were significantly higher in patients with parasitemia than in healthy asymptomatic volunteers (mean: 0.844 ± 0.290 and 0.384 ± 0.129 respectively, $p<0.001$). This MDA level was higher in primigravidea and also correlates well with parasite density ($p<0.001$). Catalase activity in erythrocytes of women with malaria did not differ statistically from that of controls. In contrast, SOD activity of patients with malaria was found to be significantly higher than that of controls (mean: 0.7899 ± 0.2777 and 0.4263 ± 0.2629 respectively, $p<0.05$). FRAP values declined, from parasitemic patients (1.4619 ± 0.6565) compare to controls (2.4396 ± 0.8883, $p<0.05$), particularly in the first and third trimester of gestation ($p<0.05$ and $p<0.01$ respectively). Finally, total erythrocyte protein concentrations of women with malaria did not differ from that of the controls. Our results suggest an imbalance between oxidants and antioxidants in pregnant women suffering from malaria, a situation which could lead to severe damage for either the mother or the fetus. Therefore, further research should be done to assess the potential benefits of antioxidant supplementation for the pregnant women suffering from malaria.

KEY WORDS

Oxidative stress, Antioxidants, Pregnancy, Plasmodium falciparum.

INTRODUCTION

Malaria is a major cause of morbidity and mortality in developing countries, accounting for an estimated 300 to 500 millions morbid episodes and 2 to 3 millions deaths per year worldwide (1-3). More than 90% of these deaths occur in Sub-Saharan Africa, most of them are due to *Plasmodium falciparum* (2-4). In Cameroon, malaria continues to cause many disasters despite enormous investments efforts. This is attributable to the mosquito vectors of tropical Africa, which probably constitute the most powerful vectorial system anywhere available to malaria parasite. As in many other malaria-endemic regions of sub-Saharan Africa, the most vulnerable groups are children under five-years of age and pregnant women (5, 6). An estimated 25 million women become pregnant in malaria-endemic areas of sub-Saharan

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Africa, with over 10,000 maternal and about 200,000 infant deaths per year as a result of *P. falciparum* infection (7, 8). Pregnant women experience lowered immunity to malaria. Malaria suppresses responses to immunogens, and placental malaria impairs materno-fetal antibody transfer, which potentially reduces the benefits of maternal immunization strategies (9-11). This is particularly frequent and severe in primigravidae (10, 11). Also, parasites-infected red blood cells (IRBCs) sequestration in the placenta is a key feature of infection by *P. falciparum* during pregnancy and is frequently associated with severe adverse outcomes for both mother and baby such as spontaneous abortion, preterm delivery, low birth weight and infant death, as well as severe anemia for both mother and infant (12-14). In Africa, 5-10% of pregnant women may develop severe anaemia (defined as haemoglobin < 70g/l or < 80g/l) (14, 15). One of the major reasons for development of malarial anemia seems to be oxidative stress (16, 17).

During malaria as in many other infections, the antigenic stimulation activates the immune system of the body thereby causing release of reactive oxygen species (ROS) as an antimicrobial action. In addition to host's immune system, malarial parasite also stimulates certain cells in production of ROS thereby resulting in haemoglobin degradation (16, 17). Important to note in this context is the fact that ROS are toxic molecules that the body antioxidant regulation system can neutralize at the cellular level through enzymes (Figure 1) or scavengers such as vitamins A, C and E (19).

Oxidative stress occurs when levels of reactive oxygen species (ROS) overwhelm the body's antioxidant defence system that regulates their production. This is a condition in which the elevated levels of ROS damage cells, tissues or organs (20). Hence, this paper reports on the role of oxidative stress on the antioxidant defence of the body in the pathogenesis of malaria as well as the implication of the changes on the outcomes of malaria in some Cameroonian pregnant women.

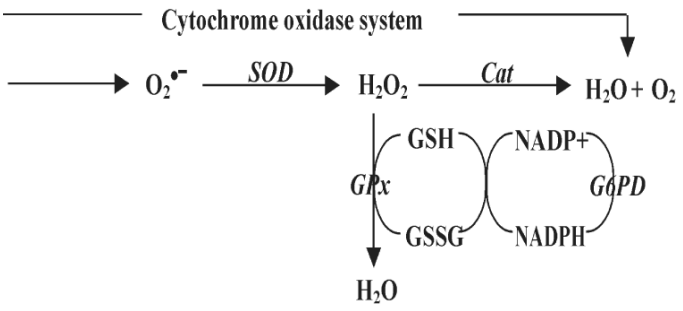


Fig1: Enzyme systems to bypass reactive intermediates released during reduction of molecular oxygen (18)

MATERIALS AND METHODS

This study took place at Douala, the most populous town in Cameroon with reportedly and constantly highest prevalence of malaria infection in the country. Douala is an area with highly seasonal transmission and malaria infections are identified more frequently in the dry season (21).

Out of the 486 pregnant women that accepted to participate in the study, 171 were excluded because they did not meet the inclusion criteria. So therefore, only 315 paucigravidae and multigravidae in the first, second and third trimester of pregnancy were selected with 159 (50.48 %) of them aged between 16-44 years that had malaria and 156 (49.52 %) controls (without malaria) aged between 18-42 years. All these women attended Laquintinie Hospital or District Hospital of Deido either for routine prenatal consultation or with clinical symptoms of malaria. After written inform consent was obtained, women were diagnosed on the basis of clinical symptoms and the analysis of thick and thin Giemsa-stained blood films for the number of parasites per 200 red blood cells. Slides were considered negative if no parasite was found in 100 fields on the thick film.

Women were matched for their BMI (Body Mass Index), term and parity. Women with established medical risk factors for oxidative stress such as AIDS, diabetes, tuberculosis, smoking and alcohol consumers were excluded from the study. At the time of enrollment, a venous blood sample was collected by venipuncture into heparinized-tubes shielded from bright light, aliquoted and stored in cryotubes at -20°C. The recovered erythrocytes were washed two times in a NaCl 0.9% solution, then aliquoted and stored at -20°C until analysis. Temperature, height and weight of Test and Control subjects were also recorded.

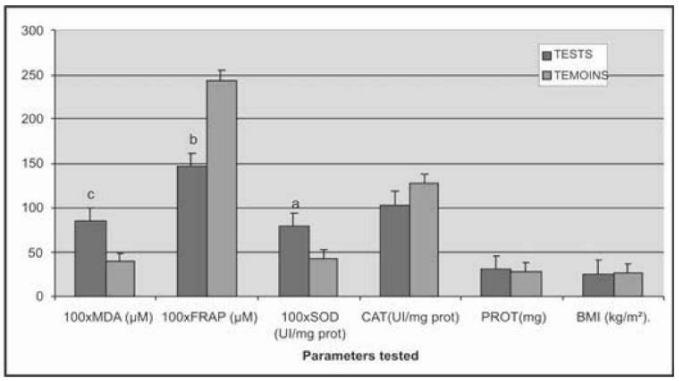
Plasma total lipids peroxides were quantified by Satoh's method also known as thiobarbituric acid method (22). Erythrocyte superoxide dismutase activity was measured by the method of Misra and Fridovich (23). Erythrocyte catalase activity was assessed by the method of Beers and Sizer (24). FRAP (Ferric Reducing Ability of Plasma) method was used for the determination of the total antioxidant capacity according to Benzie and Strain (25).

Statistical analysis was carried out by using Student's paired and unpaired 't' test. The $p<0.05$ was considered significant. Software used: SPSS 10.0 and Excel 2003 for Windows.

RESULTS

Several differences were observed between the biochemical parameters tested when comparing the Test and Control groups in the experiment (Figure 2). Plasma total lipid peroxides is raised in malaria pregnant patients as compared to controls, regardless of the trimester of gestation ($p<0.05$) (Table 1). This increase of plasma MDA concentration is highly correlated with parasitemia and it was markedly observed with young paucigravidae aged between 16 and 27 years (Table 2). We also noticed that this increase is more important during the first pregnancies than with subsequent pregnancies ($p<0.001$) (Table 3).

Fig 2: General variation of the parameters tested



[c]($p<0.001$), [b]($p<0.01$), [a]($p<0.05$) compared to controls

The enzymatic activity of SOD is more elevated in pregnant women with malaria in the first and second trimester of gestation as compared to the healthy controls. Otherwise, we noticed that, the difference is more appreciable during the first trimester of gestation ($p<0.01$) than in the second trimester

Table 1: Variation of the parameters tested with term Parameter tested (Mean±SD)

		TERM (Trimester of gestation) 1st	2nd	3rd
MDA (µM)	Test	0,725±0,155[a]	0,628±0,359[a]	0,689±0,338[a]
	controls	0,408±0,172	0,362±0,115	0,383±0,088
SOD (UI/mg of protein)	Test	0,609±0,342[b]	0,501±0,242[a]	0,483±0,187
	controls	0,387±0,372	0,448±0,197	0,425±0,187
Catalase (UI/mg of protein)	Test	112,89±17,62	117,904±21,63	104,086±16,26[a]
	controls	129,21±30,076	125,59±37,356	127,32±47,112
FRAP (µM)	Test	1,7185±0,6844[a]	1,9935±0,7382	1,6475±0,5209[b]
	controls	2,2641±0,7128	2,1512±0,6572	2,5875±1,1599

[c]($p<0.001$), [b]($p<0.01$), [a]($p<0.05$) compared to controls; SD = Standard Deviation

($p<0.05$) (Table 1). This elevation in the enzymatic activity of SOD is observable with young paucigravidae aged between 16-27 years (Table 2).

We noticed a decrease of catalase activity in the pregnant women with malaria than in healthy controls during the third trimester of gestation (Table 1). The level of plasma total antioxidant capacity (TAC) is low in pregnant malaria patients compared to controls regardless of gestations period ($p<0.01$) (Table 3). This decrease of TAC is observable in the first and the third trimester of gestation but the decrease was more during the 3rd trimester ($p<0.01$) than the 1st ($p<0.05$) (Table 1).

DISCUSSION

The increase lipid peroxides was an ultimate toxic effect of raised reactive oxygen species production by the immune

Table 2: MDA concentration, SOD and Catalase activities variations with age and parasitaemia

		Paucigravidae (0,1 or 2 gestations)		
Age groups (Years)	Median parasitaemia Parasites/µl (Range)	MDA (µM)	SOD (UI/mg of protein)	Catalase (UI/mg of protein)
16 - 21	18,760 (6000-56000)	0.872 ± 0.266[c]	0.5652 ± 0.2216[a]	116.327 ± 19,422
22 - 27	25,392 (1200-82000)	0.843 ± 0.246[c]	0.5794 ±0.3261[a]	113.695 ± 18.263
28 - 33	7,200 (800-13000)	0.656 ± 0.128	0.4857 ± 0.2038	98.343 ± 18.287
34 - 39	12,600 (1600-22000)	0.764 ± 0.162	0.5328 ± 0.2153	122.018 ± 19.383
40 - 44	1340 (1200-1800)	0.574 ± 0.138	0.4738 ±0.2367	102.327 ± 18.166

MDA, SOD and Catalase values in the table are written as Mean ± SD; [c]($p<0.001$), [a]($p<0.05$) compared to controls; SD = Standard Deviation

Table 3: Variation of the parameters tested with parity (Mean±SD)

Parameter tested		PARITY (Number of gestations) 0	1-2	3 or more
MDA (mM)	Test	0,744±0,163[c]	0,773±0,424[c]	0,764±0,282[a]
	controls	0,361±0,111	0,443±0,147	0,428±0,141
SOD (UI/mg of protein)	Test	0,5556±0,331 [a]	0,5692±0,2079[a]	0,4363±0,1807
	controls	0,4547±0,2813	0,3693±0,2503	0,3895±0,2337
Catalase (UI/mg of protein)	Test	103,5075±18,452[a]	121,261±19,826	123,615±16,457
	controls	125,4088±36,3263	133,722±49,041	112,055±19,837
FRAP (mM)	Test	1,6955 ± 0,7069[b]	1,8492 ± 0,606[b]	1,6145 ± 0,6263[b]
	controls	2,3062 ± 0,8529	2,4016 ± 1,0836	2,3293 ± 0,619

[c](p<0.001), [b](p<0.01), [a](p<0.05) compared to controls; SD = Standard Deviation

system of the body, as well as synchronised release of $O_2^{\bullet-}$ during haemoglobin degradation by malarial parasites.

It has been demonstrated that *P. falciparum* infection during pregnancy is frequently associated with sequestratrion of infected red blood cells (IRBCs) in intervillous space of the placenta, leading to a pathology known as placental malaria. The presence of IRBCs frequently induces the infiltration of inflammatory-type cells into the placenta, where they cause pathological alterations (26). It has been also shown that *P. falciparum* trophozoite infected human red cells produce H_2O_2 and OH^- radical about twice as much as normal erythrocytes (17, 27). Excess H_2O_2 could also result in breakdown of heme and release of free iron ions, which in turn form OH^- through Fenton reaction (28). All these factors cause a substantial rise in lipid peroxides, leading to oxidative stress in malaria.

Women in endemic areas become highly susceptible to malaria during first and second pregnancies, despite immunity acquired after years of exposure to malaria infection but they acquire a strong immunity with an increasing number of pregnancies (29). Younger maternal age is an independent risk factor for malaria in pregnancy (i.e. young mothers are at greater risk of malaria and its adverse effects than older primigravidae or multigravidae, respectively) (15). This suggest that in addition to the parity-specific immunity that is acquired through consecutive pregnancies, age-associated immunity also plays an important role in controlling the infection during pregnancy in areas of high and stable transmission (6). The pronounced enzymatic activity of SOD in pregnant women suffering from malaria make us speculate that patients still in beginning of stress. SOD indeed is an inducible enzyme and its activity depends on the evolution stage of stress (30). It has been shown that in addition to utilization of host SOD, *P. falciparum* also synthesizes its supplementary specific SOD isoenzyme, which is cyanide insensitive, unlike that of human SOD (31) and therefore increasing the concentration of the enzyme then its activity. This spontaneous increase of the enzymatic activity in beginning of stress would be therefore adaptive to the fast and massive free radical formation caused by oxidative stress (32). The decline of catalase activity shows that this enzyme could act as oxidative drugs that mediate parasitic clearance. In fact, malarial parasites are sensitive to oxidative stress and utilize the host's enzyme catalase to counteract the oxidative damages (33).

This decline of catalase activity could be potentially linked to the fact that even if catalase may not be the main protective enzyme involved in the protection against oxidative stress; it may become critical under the higher oxygen tensions conditions encountered in malarial oxidative stress (34). The decrease of plasma total antioxidant capacity in malaria patients compared to the controls is in agreement with the brief "momentous" increase in oxidative stress. Due to their synergistic functioning, the non enzymatic antioxidants depresses in concert with the brief "momentous" increase in total lipid peroxides (35). Then, the antioxidants degraded by malarial parasites to derive amino acids and other vital molecules, can not be replenished by red blood cells due to lack of protein synthesis which might be one of the reasons behind overall decrease of the body antioxidant compounds (36).

We conclude that oxidative stress has a dual function. First, when highly induce it is involved in tissue damage and secondly it may as well contribute in malaria parasite destruction. These mechanisms lead to the decrease of the antioxidant capacity of the body therefore reflecting the severity of *P. falciparum* malaria. In addition, our data show that primigravidae are more susceptible to severe malaria than multigravidae in our study population. Finally we showed that women at their first pregnancy are more susceptible to the morbidity of *P. falciparum* malaria than those with multiple pregnancies. A longitudinal survey including an antioxidant supplementation after adequate treatment against the malaria parasite should bring more insight on the role of oxidative stress in the pathogenesis of malaria in pregnancy.

ACKNOWLEDGEMENTS

This paper is dedicated to late Professor Gabriel LANDO who initiated this work. This paper would not have been possible without his untiring work during the development and implementation of the study.

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