The Possible Role of Diet in Mitigating Oxidative Stress Induction during Plasmodial Infection

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Abstract

The effect of diet on the generation of oxidative stress in two groups of plasmodial-infected persons resident in predominantly carbohydrate-dominated hinterland (group 1) and seafood-dominated coastal (group 2) eastern states of Nigeria with close cultural relationship but, substantial nutritional difference was investigated. Selected volunteers (male malaria patients and male plasmodial uninfected controls) from both groups who were fed on their respective types of diet, with restricted alcoholic drinks for a period of two months were involved in this investigation. The plasma levels of malondialdehyde (MDA), antioxidant vitamins (C and E) and the polyunsaturated fatty acids [arachidonic (20:4) and decosahexaenoic (20:6) acids] of the study subjects were photometrically assayed. Results revealed significant higher MDA levels (p<0.05) in plasmodial-infected patients from groups 1 and 2 states when compared with those of their respective controls. However, the malaria patients from group 1 states manifested significantly higher MDA level (p<0.05) but significantly reduced plasma levels of the antioxidant vitamins and polyunsaturated fatty acids (P<0.05) than those of group 2 plasmodial-infected patients. These results seem to implicate the difference in diet in mitigating oxidative stress during plasmodial infection.

Keywords: Malaria, Oxidative stress, Diet

Introduction

Malaria is one of the widely distributed tropical diseases that pose the most serious, life–threatening public health problems in many African countries. (Golenser and Chevion, 1993). The disease presents a spectrum of acute haemolytic anaemia, relapsing fever, severe weakness, hypoglyceamia and high mortality rate among the populations of Nigeria (Greenwood, 1996; Felger et al, 2003). Plasmodial parasite, exerts oxidative stress on its host's erythrocyctes (Hunt and Stock, 1990) by generating reactive oxygen species (ROS) (Eze, 1991; Mishra, *et-al*, 1994) through oxidative immune reactions which are part of immune response to plasmodial infection (Golenser and Chevion, 1993). Reactive oxygen species could also be generated by the host's phagocytes, activated by the plasmodial antigens (Ockenhouse and Shear, 1984). Increased intracellular oxidative stress which could be generated by excess ROS originating from parasitized erythrocytes or by polymorphonuclear nutrophils (Buffiton *et al*, 1988) or plasmodial antigen activated macrophages (Golenser and Chevion, 1993) could cause serious damage to the cells or tissues of the host or to the intracellular plasmodial parasite (Hunt and stocker, 1990). The damage could be targeted to the bilayer of the host erythrocytes (Mishra *et al*, 1994) thereby causing anaemia and severe weakness usually associated with malaria or at the membrane of the intercellular plasmodial parasites. However, the host can protect itself against the deleterious effects of excess ROS by mobilizing a set of enzymatic antioxidant defence systems or endogenous antioxidant vitamins (Clark and Hunt, 1983). But in overwhelming circumstances, higher oxidative stress is generated which could mediate lipid operoxidation process (Jiankang, *et al* 1997).

Although lipoperoxidation process has been known to be generated in plasmodial infections (Ogugua and Eze, 2001, Uzoegwu and Onwurah, 2001) and other infections (Nwaka, 2004) in the eastern states of Nigeria, the possible effect of diet on the severity of these infections has not been investiaged. The main aim of this study therefore is to investigate the mitigating role of diet on oxidative stress generated in malarious state in two populations of eastern states of Nigeria with different dietary patterns since the immune defence processes of two such populations could be affected by such dietary difference (Jensen, *et al*, 1984).

Materials and Methods

Study area: This study was carried out in the populations of Abia, Anambra, Enugu and Imo states, located at the hinterland of the old eastern region of Nigeria (group 1 states) on one hand, and those of Akwa Ibom, Bayelsa, Cross River and River State, located at the oil-rich coasts of the Atlantic ocean (group 2 states) on the other (Fig. 1). Although these populations have close cultural relationship, substantial differences in dietary pattern, vegetation and climate exist between them. The coastal residents naturally feed mainly on the diet consisting crayfish, periwinkle, shrimp, lobster, sea cucumber, crab, snails, green vegetables and some quantity of carbohydrate foodstuff as compared to the carbohydrate-dominated diet of the hinterland residents.

Study subjects: A total of 3588 male and female volunteers of all ages (group 1 states – 2202, group 2 state – 1386) were initially tested for plasmodical infection by parasitologic method of diagnosis. Two hundred (200) male volunteer farmers, traders, and artisans (30 – 45 years old) were selected from the tested persons in each group of states on the basis of accessibility, willingness to be restricted from alcohol and comply with their natural feeding habits.

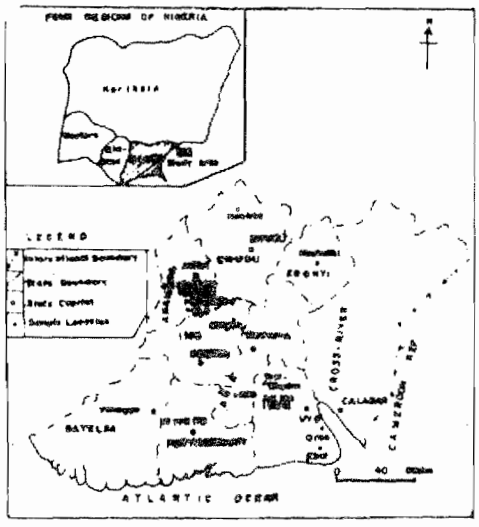

Fig. 1: Map of south eastern Nigeria (study area)

The selected volunteers were monitored for two (2) months for their respective feeding habits and plasmodial infection and their plasma levels of lipid peroxidation index, antioxidant vitamins (C and E) and polyunsaturated fatty acids (oxidative profile) were assayed during the monitoring. Only volunteers with *Plasmodium falciparum* (P.f) and *plasmodium malariae* (P.m) monoinfections, glucose–6–phosphate dehydrogenase (G6PD) non-deficiency and those not suffering any other ailment by the time blood was collected from them were involved in the oxidative profile assays.

Collection of blood samples: Blood samples (5 ml) were collected from the selected volunteers by venipuncture into sample tubes containing heparin as anticoagulant. Plasma was prepared from about 4.5 ml of the blood by centrifugation (Hettich bench centrifuge) at 3000 × g for twenty minutes. The top layer (plasma) was either used immediately or stored in deep freezer (– 4 °C) in aliquots of 200µl until used.

Malaria diagnosis: The volunteers from both hinterland (2202) and coastal (1386) states were first subjected to intense clinical examination for possible malarial or other disease symptoms. The presence of plasmodial parasites was then demonstrated by microscopic examination of Giemsa-stained thin and thick blood films. The presence of malaria parasite in a microscopic field is regarded being positive for malaria.

Parasite density determination: Based on individual count, the geometric mean of parasite density (GMPD) per microlitre of blood was determined for all the *P.f* and *P.m* monoinfections by WHO (1991) method.

Lipid peroxidation assay: Lipid peroxidation was assayed according to the spectrophotometric method of Albro, *et al* (1986) as modified by Das, *et al* (1990). The principle guiding the assay is based on the complexing, in acid pH, of thiobarbituric acid with malondialdehyde the main aldehydic end-product of lipid peroxidation (Jiankang, *et al*, 1997) to form a red chromogen that absorbs maximally at 532 nm.

Determination of plasma level of vitamin C and E: Plasma levels of vitamin C of 186 and 162 MP-positive subjects and 82 and 72 MP –negative controls in groups 1 and 2 states respectively were determined according to the method reported by Thurnham, et al (19s90). Plasma vitamin E level was evaluated in 160 and 113 MP-positive individuals and 92 and 76 MP- negative control subjects in group 1 and 2 states respectively according to the method described by Baker and Frank (1968).

Determination of plasma content of polyunsaturated fatty acids: Plasma contents of polyunsaturated fatty acids, Arachidonic acid (ARA, 20:4) and Docosahexaenoic acid (DHA, 22:6) were extracted according to the method of Penchant, *et al*; (1989) and their concentrations determined according to the method of Delmas-Beanvieux *et al*, (1995) Reference fatty acids (Sigma, St. Louis, U.S.A) were used. The fatty acid concentrations of individual samples were expressed as a percentage of the total extract.

Results

Prevalence of plasmodial infection: The incidences of plasmodial infections and the geometric mean parasite densities for the two populations are displayed in Table I. The overall incidence of malaria in group 1 population (47.73%) is less than that of group 2 population (53.32%). *Plasmodium falciparum* was the major cause of malaria disease in the two populations (Fig. 2). Although the incidence of malaria is higher in group 2 than that in group 1 populations, the geometric mean parasite density of the former (GMPD, 618.65/µl) is significantly lower ($p<0.05$) than that of the later (715.52/µl). Clinical observation of the plasmodial infected patients showed that the group 1 patients presented less severe symptoms of malaria than the patients of group 2 population.

Lipid peroxidation: The results of the malondialdehyde assay of plasmodial parasite infected and uninfected individuals showed that uninfected controls exhibited some concentrations of plasma MDA. But the differences between the mean plasma MDA concentration of MP- positive subjects and MP-negative controls in the two populations were significant for group 1 ($p<0.05$) and for group 2. ($p<0.01$) (Table 2) indicating enhanced lipid peroxidation in MP-positive subjects. Furthermore, higher mean plasma MDA concentration was revealed for patients infected with *P.f* species than those infected with *Plasmodium malariae*.

Antioxidant vitamins C and E: Both populations showed significant reduction ($p<0.01$) in the plasma vitamin C and E concentrations in MP-positive

Table 1: Incidence of plasmodial parasite infections and GMPD in the two groups of populations (No. in bracket is the percentage infection)

Group	Sampled Population	Number of People Infected				GMPD
		p.f	*p.m*	Mixed	Total	
Group 1 (Hinter land)	2202	1040 (47.22%)	7 (0.32%)	4 (0.18%)	1051 (47.73%)	715.52/ml
Group 2 (Coastal State)	1386	739 (53.32%)	10 (0.72%)	3 (0.22%)	752 (54.26%)	618.65/ml
Total	**3588**	**1788 (49.83%)**	**17 (0.47%)**	**7 (0.20%)**	**1805 (50.31%)**	

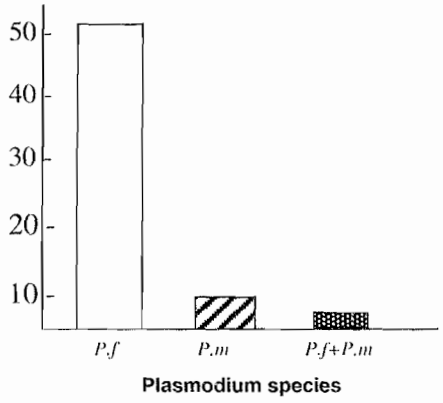

Fig 2: Percentage Infection of *Plasmodium falciparum* (□), *Plasmodium malariae* (▨) and mix P.f +P.m (▩) in the study area

patients than in MP- negative subjects, indicating the consumption of the endogenous antioxidant during malarial state (Table 3). Results further showed that MP-positive coastal residents manifested significantly higher vitamin concentrations ($p<0.02$) than their counterparts in the hinterland population.

Polyunsaturated fatty acids concentrations: The plasma concentrations of arachidonic acid (20:4) and docosahexaenoic acid (22:6) of malaria patients decreased significantly ($p<0.05$) when compared with the levels in MP-negative patients of the two populations. (Fig 3)

Discussion

The possible role of diet in reducing oxidative stress in malarious state as investigated in this study offered an opportunity for a revelation of higher prevalence of malaria, caused mainly by *Plasmodium falciparum, in* coastal than in the hinterland populations. This revelation is understandably justified by the swampy nature of the coastal states which naturally favours the breeding of much more mosquitoes than the drier hinterland area. The presence of more infected plasmodia vectors in an area could subsequently favour more inoculations of the coastal inhabitants with plasmodial parasites than the residents of the hinterland states. However, it was surprising that the geometric mean of parasite density (GMPD) of the coastal population was significantly less ($P<0.01$) than that of the hinterland population despite the higher malaria prevalence in the former. The relative lower GMPD value could invariably indicate some element of protection against malaria. This protection is supported by the less severe malarial clinical symptoms observed among the coastal than among hinterland populations. Since the costal group 2 residents feed mainly on fishes that could be rich in polyunsaturated fatty acids and other seafood stuff which are known to contain vitamins, it is logical therefore to implicate antioxidant-rich diets, such as was eaten in the coastal study population because fish-oil products and other seafood stuffs, rich in unsaturated fatty acids provided protection to vitamin E-deficient mice against chloroquine-resistance malaria strain (Lavander *et.al*,1989) and also pro-oxidant diets, supplemented with fish oil containing high concentrate of antioxidant vitamins, strongly protected mice against parasite invasion (Lavander, *et al;* 1990). It is not therefore surprising that, coastal states residents could be more protected against malaria than the inhabitants of the hinterland states whose diet is dominated by carbohydrate. The protection given to the coastal state inhabitant by the seafood they consumed, as implied above, is consistent with the significantly lower mean plasma MDA concentration observed among the coastal than among the hinterland populations. High MDA level is suggestive of the generation of more excess reactive oxygen species (ROS) in a system, which could be available to mediate more lipoperoxidation process to produce more end-products such as MDA. This study therefore suggests lower lipoperoxidation process among the costal population during malaria attack. However the availability of large amounts of antioxidant systems could invariably minimize the extent of the lipid peroxidation and consequently, the level of MDA by detoxifying excess ROS (Delmas–Beauview *et al* 1995). The heightened plasma MDA level consistently observed in malaria patients in this study is consistent with the high lipoperoxidation process reported during malaria disease (Das, *et al*, 1990; Mohan, *et al*; 1992, Delmas Beauviex, *et al*; 1995). More lipid peroxidation is likely to be generated during malaria attack by excess ROS which are known to be high during malaria disease (Thurham, *et al*, 1990). The detection of appreciable quantity of MDA in MP – negative controls, even though normally plasma lipids and lipoproteins are naturally well protected from peroxidations process (Halliwell and Guttridge, 1992) could be explained by the production of MDA in healthy subjects during tissue metabolism (Guttridge and Trickner, 1978).

Table 2:. Mean plasma MDA conc. in MP-negative and MP-positive subjects

	Subjects Status Subjected	Mean MDA conc. Group 1	Mean MDA conc. (nmol.) Group 2
A.	*P.f*-Negative	4.38 ±0.02(56)	4.139 ±0.05(45)
B.	*P.f*-Positive	5.94 ±0.03(514)	5.45 ±0.03(501)
C.	P. M-Positive	5.08 ±0.02(3)	4.87 ±0.04(3)
	Difference (B-A)	1.56 ($p < 0.05$)	1.32 ($p < 0.01$)
	Difference (C – A)	0.70	0.74
	GMPD	**715.52/µl**	**618.05µl.**

(Number in bracket is the Number tested)

Table 3: Mean plasma concentrations of vitamins C and E in MP-negative and uninfected individuals

Subject status	Vitamin C Level (mg/100 ml)			Vitamin E Level (µG/L)		
	Group 1 States	Group 2 States	Group Diff.	Group 1 States	Group 2 States	Group Diff.
P.f- ve	1.42 ±0.12 (83)	1.52 ±0.1(72)	0.1 (p>0.01)	11.73 ± 0.4 (92)	12.33 ±0.6 (76)	0.57 (p>0.05)
P.f+ve	0.74 ± 0.03(186)	1.16 ± 0.05(162)	0.42 (p<0.02)	9.92 ±0.4(160)	10.53 ± 0.5(113)	1.11 (p<0.02)
Difference between infected and uninfection	0.68 (P<0.01)	0.36 (P<0.01)		1.81 (P<0.01)	2.0 (P<0.01)	

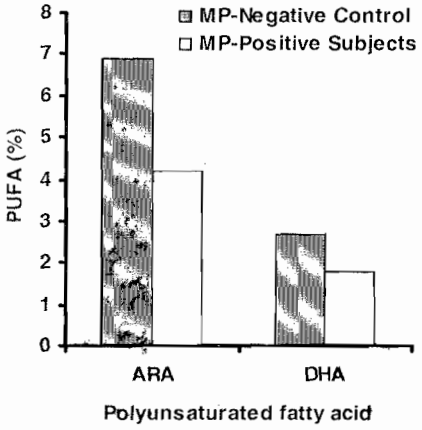

Fig 3: Arachidonic Acid (ARA) and Decosahexaenoic Acid (DHA) Profile in Subjects Infected and Uninfected with Plasmodial Parasite

The lower MDA level observed in both MP-infected and uninfected subjects from coastal than those from hinterland populations, possibly suggests lower lipoperoxidation process in this population perhaps due to the higher antioxidant vitamin levels available to detoxify excess ROS. The enhanced MDA level during *P. falciparum* than during, *P. malariae* infections as observed in this study could be attributed to the selective infection pattern of *P. malariae* which infects only old red blood cells (RBCs) while *P. falciparum* infects both old and young RBCs (Wellde, *et al*, 1971). Since *P. m* parasite infects fewer RBCs than *P. f* parasite, it is logical to conclude that more lipid peroxidation process will occur in the later than in former infections. The presentation of more virulent and more severe symptoms in *P.f* infection lends credence to the above conclusion. Differences in the pathogenic patterns of these two plasmodial species could ostensibly introduce such differences in the plasma MDA levels of the two groups of patients. The reduced plasma vitamins C and E levels observed in MP – infected subjects are consistent with the similar results reported in Thiosand (Das, et al, 1990) and in Califonia, (Chiu, *et al*, 1982). Hence the difference is therefore not surprising given that in normal metabolic conditions, the oxidation of plasma lipoproteins is protected by a host of endogenous antioxidant system (Alphro and Leinonen, *et al.*, 1999) including antioxidant vitamins (Guttridge and Trickner, 1978). The protection implied above, could be supported by the loading, in vitro, of *Plasmodium falciparum* – parasitised erythrocytes with antioxidant vitamins, possibly to protect the cells against oxidation (Mishra, et.al 1994). The antioxidant properties of vitamin C and E (Stahl and Seis, 1997) are known to provide protection against ROS during the acute phase of malaria by detoxification (Delmas – Beauvieux, *et al*, 1995). However, loss of appetite and poor absorption characteristics of malarious state could contribute to this reduced plasma antioxidant vitamins.

The enhanced MDA level and reduction in the plasma concentration of vitamins C and E in malarious state are indicators of increased oxidative stress during the progress of malaria. Although the coastal MP – infected patients presented less severe symptoms and higher incidence of malaria when compared with other hinterland counterparts, their plasma vitamin C and E levels were higher. These findings could be due to differences in the dietary constituents of the two populations. However, genetic and other factors may well contribute to such differences. But the results could invariably indicate the importance of diet rich in antioxidant vitamins in the management of malaria and perhaps other diseases. The significant reduction, of plasma levels of ARA (20:4) and DHA (22:6) ($p<0.05$) in subjects infected with *P. f. p*arasite is consistent with the similar report of Delmas -Beauvieux, *et al* (1995) in a population

infected with plasmodial parasite, suggesting the mitigating role of diet rich in antioxidants in the reduction of oxidative stress in malaria disease.

In conclusion the enhanced plasma MDA level, the reduced plasma levels of antioxidant vitamins and the reduction in polyunsaturated fatty acids observed in this study seem to suggest the generation of oxidative stress during malaria disease which appear to be reduced by the intake of diet rich in antioxidant vitamins. Presently an investigation on the possible reduction of oxidative stress during plasmodial infection with dietary antioxidant vitamin supplimentation is ongoing.

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