

Studies on Mean White Blood Counts in Malaria Infected Mice Treated with Antioxidants

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Abstract

A parasitologic evaluation of blood samples of 2000 symptomatic malaria patients (1000 males and 1000 females) in some health facilities of Enugu metropolis was conducted to determine the prevalence of prevalence of plasmodium (P) species, seasonal pattern; sex and age specific pattern with special reference to pregnant women. Holistic management of malaria with antioxidant Vitamins A.C.E'. In animal model was employed. Gnotobiotically reared male Swiss albino mice were inoculated with a standard dose of *p. berghei*, about 10^7 parasitized erythrocytes through intraperitoneal route. Treatment was administered according to body weight of mouse through intraperitoneal route, observing the lethal dose of Vitamins through the route. The blood was evaluated parasitologically. Plasmodium species encountered in patients showed significant difference ($P < 0.05$) in the distribution *P. malariae*, *P. falciparum*. For seasonal pattern, 830 (67.9 percent) was recorded coincide with rainy seasons while. 390 (50.1 percent) was recorded in dry seasons. A prevalence of 880 (88.0 percent) was recorded in males and 340 (34.0--percent) in females. Prevalence by gestational age in pregnant women showed 27 (9.6. percent) in second trimester and 80 (20.5 percent) in the trimester. There was a highly significant decrease ($p < 0.0001$) in parasitaemia, consequent to total clearance in all mice treated with antioxidant vitamin. A hundred (100 percent) mortality was recorded in positive untreated control group. Post mortem examinations revealed haemorrhagic lesions at the lower part of the brain. There was an effective treatment by orthomolecular approach.

Keywords: Prions; Neurodegenerative Diseases; Cancer

Introduction

Four parasitic protozoa of the genus plasmodium (P) which include *P. ovale*, *P. vivax* *P. malariae* and *P. falciparum* cause human malaria. *Plasmodium falciparum* cause the most severe morbidity and mortality, are found throughout tropical Africa, Asia and Latin America [1]. All life four species are transmitted to man through the bite of an infected female. *Anopheles* mosquito species of *gambiae* complex, *funestus* and *darling* [2]. Other less common routes of infection are through blood transfusion and Maternal-fetal transmission. Malaria remains an enormous international medical issue, being one of the commonest, oldest and extensively researched tropical diseases of our time, with high morbidity and mortality rates. Globally, 300 - 500 million deaths occur annually. Ninety percent of deaths each year come from rural Sub Saharan African [3]. All age are affected. Malaria contributes to maternal deaths. Complications of malaria include cerebral malaria, pulmonary oedema, rapidly developing anemia, vascular obstruction. Black-water fever, hyperpyrexia, algid malaria, severe gastroenteritis, nephritic syndrome, tropical splenomegaly and low birth weight in babies whose mothers have heavy malaria parasitization of the placenta [4].

There is increasing resistance of parasite species to some of the existing drugs [5]. Drug resistance stresses the loss of response of parasite to the effect of the active compound. Then, effectiveness of the drug on the parasite depends on the parasitaemia and the status of the host's immunity. Moreover, it is conceivable that some nutritional and other factors in the host play an important part in the response of the parasite to the drug [6]. Stress condition enhances relapse of latent inhibited malaria parasites in the state of depressed immune system or by a failing off in immunity brought on by physiological shocks as in exhaustion, childbirth, operations and many other conditions [7]. There is evidence from animal studies that marked vitamin A deficiency increases the severity of malaria [8,9]. The release of iron and copper ions (catalysts) from ruptured erythrocytes in malaria infection in man catalyzes lipid peroxidation of polyunsaturated fatty acid from dietary fats and oils and those in the lymphatics, especially the thymus gland to cause excess proliferation of free-radicals. The condition gives rise to inflammation, fever, pain, destruction of cell membranes and nuclear materials [10-13]. At this juncture, there is an intermittent rise in temperature as the new generations of merozoites are liberated into the blood stream. It is characterized clinically in malaria by paroxysms of cold stage (rigor and chill), hot stage (pyrexia or high fever) and sweating stage (defervescence).

Chelation of unbound catalyst by administration of increased amount of antioxidant vitamins stops free-radical proliferations, febrile conditions and parasitaemia [14,15]. They strengthen the immune system for better body defence mechanism, thereby enhancing phagocytosis [16].

Aim of the Study

The study aimed at determining the role of antioxidant vitamins in the management of plasmodium infection using animal's model.

Materials and Methods

Host animals

Male Swiss albino mice aged six to eight weeks were used. They weighed between 18 and 22g, gnotobiotically reared and purchased from the Department of Pharmacology and Toxicology, University of Nigeria Nsukka (UNN).

Housing and feeding

The animals were housed in the experimental room of the animals house at UNTH premises belonging to the college of medicine, University of Nigeria Enugu Campus (UNEC). The animals were maintained in a conventional unit of clear plastic boxes with sawdust bedding. Each box represented a group which consisted of 5 mice. They were 6 groups of mice A-F, housed in their respective boxes, at 24#1°C, and 10 hour light (9 am - 6 pm); 14 hour dark (7 pm - 8 am) cycle was maintained throughout the experimental period.

They were fed with normal mouse cubes purchased in bags from Pfizer Product Limited, P.M.B. 2111, Ikeja, Nigeria. The standard normal mouse diet (NMD) contained protein (21.0%), fat (3.5%), and Carbohydrate (70.0%). Other constituents in the Pfizer cubes which included vitamin premix, wheat middlings, and oyster shell, Brewer's yeast and maize were not indicated of their percentages. The mice were fed with diet and water and libitum. The ventilation was good and the environment also was neat and dry.

Parasite '*plasmodium berghei* Anka Strain'

Plasmodium (P) *berghei* was maintained in the pharmacology Department, College of Medicine, Lagos University Teaching Hospital (LUTH) Lagos, by blood passage into Swiss albino mice. Five male swiss albino mice were transported from the animal house, Pharmacology Department, College of Medicine University of Nigeria Enugu Campus (IJNEC) in a cage to LUTH. They were fed with a standard diet and water and libitum. A standard dose of parasitized red blood cells (RBC_s) was inoculated by intraperitoneal route into native animals.

The blood was diluted with 0.9% normal saline w/v aqueous to give 10⁷ parasitized erythrocytes in each inoculum. The infected animals forms LUTH were used as the infected stock, with which the animals for the study were infected at UNEG. The animals used for the study were allowed to acclimatize for four days before they were infected.

Procedure of infection and treatment with antioxidant vitamins

Methodology

Five mice were housed in each cage. Cages were grouped into six, A, B, C, D E and F. the first groups A, B, C, D and E were infected with *P. berghei*, while the last group F had random sampling of uninfected blood, all given by intraperitoneal route. The first four groups A, B, C and D and treatments, while the last two groups E and F had no treatments and were used as positive and negative control groups respectively (Table 1).

Antioxidant vitamin (Vit) A and C injections and Vit. E tables, ground and dissolved in injection water according to milligram per ml were administered by interperitoneally (i.p) route into groups A to D mice. Groups E and F mice had no treatment (Table 2). The antioxidant vitamins used were potent, and met the standards laid down by the international pharmacopoeia or the National Pharmacopoeia of the country. Three hundred milligram (300 mg) base of Vit. A., 500 mg base Vit. E and 500 mg base Vit. C, all calculated according to milligram per kilogram of body weight (B.W) of mice were administered on day 0, 1 and 2. Vitamins A and E were given twice daily while vitamin C was given once daily and gently and slowly.

The groups were treated as follows Group A (mice) had antioxidant Vitamin A, Group B (mice) was given antioxidant vitamin C., Group C (mice) had antioxidant vitamin E, and Group D (mice) had a combination of antioxidant vitamins A,C and E (Table 1).

Group 5 mice/ group	Group passed with <i>p. berghei</i> infected blood	Group Passaged with ubifected bolloed	Group Treated with Antioxidant vitamin(vit.)	Positive control Group	Negative Control Group
A	A		Vit. A		
B	B		Vit. C.		
C	C		Vit. E		
D	D		Vit. AC and E		
E	E		No treatment	E	
F	No infection	F			F

Table 1: Infection and treatment of host animals (mice) with antioxidant vitamins.

Sample collection

Collection of blood samples were carried out on day 0, 2, 4, and 6. Blood was collected from the nipped tails of the mice (after cleaning with 70% alcohol) for films white Blood Counts (WBC). The WBC was raised by the presence of infection. Collected of blood on day 0 was done before treatment in order to determine the initial level of parasitaemia in all the mice.

Parasitologic procedure

Thick films were made and stained with 10% Giemsa solution in buffered distilled or deionized water, pH 7.2 for 5 - 10 minutes.

Gently, the stain was flushed off to avoid deposit of scum over the film. Parasites count on thick film was based on the number of parasites per ml of blood or per 200 white blood cells. These were counted in relation to a predetermined number of leukocytes. An average of 8,000 Leukocytes per ml was taken as standard, despite inaccuracies due to variation in the number of leukocytes in animal model, in normal health, and greater variation in ill-health. The equivalent of 0.025ml of blood (25 per microlitre) about 100 fields and using x 7 ocular, and X 100 oil immersion objective, the number of parasites were determined. The parasite per ml or parasitaemia was noted by simple mathematical formula [17]:

$$\text{No. of parasite counted} \times 8.000$$

No. of Leukocytes counted

Results

On infection of mice, they become unwell on the third day. They moved slowly, sat hunched and shivered. They had ruffled fur. They showed locomotor disturbances with parasites. Some had poor visions ore were blind. All were moribund in the positive control group E and died between the 5th and 6th day of infection. Infect, they was a 100% mortality of the mice in the positive control group. Post mortem examinations on the lower haemorrhagic lesions at the meningeal surfaces or the lower brain surfaces. The negative control group F mice were all alive and health all through the study.

Clearance of parasitemia in Group D mice was recorded earlier than other Group. On clearance of parasitaemia, it was noted that the mice fed well, moved fast, become agile as locomotion tremendously improved. Their fur becomes normal. Their visions really improved as the reacted very sensitively to touch.

The data recorded, confirmed reductions of white blood counts in each group. The mean WBC values recorded on day 6 showed in descending order that Group A treated with Antioxidant Vitamins (AV) - A had the highest value, followed by Group C treated with AV-E, then Group B treated with AV-C and finally Group D treated with AV - A, C and E had the lowest value (Table 2). The prevalence of *plasmodium* infection was represented graphically as lowest in Group D nice (Figure 1).

The positive control Group U mice which was infected, and received no treatment, died after the count on day 2, consequently terminated the subsequent counts (Figure 1). Group F negative control which had no infection and had no treatment maintained almost the same values between day 4 and day 6 (Figure 1).

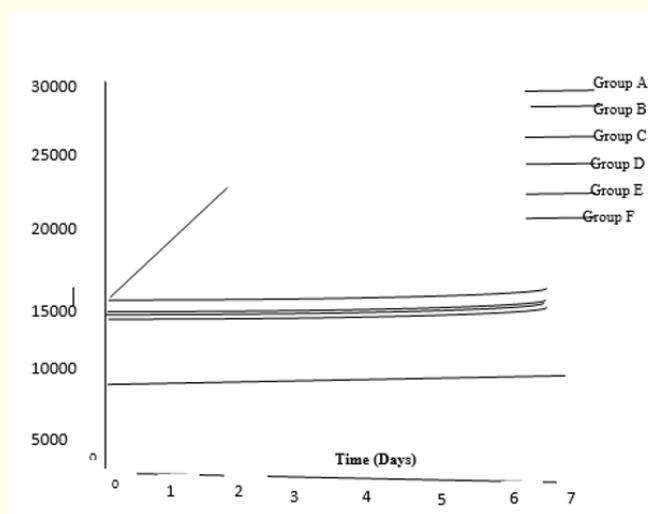


Figure 1: Mean white blood count (WBC/mm³) in mice of various groups. A highly significant decrease (< 0.0001) in the mean WBC throughout the study.

Statistical analysis showed a highly significant decrease ($F = 40.94, P < 0.0001$) in the WBC throughout the study in various groups (Table 2). There was no significant difference ($p > 0.05$) observed between the mean count on day 0 and 2 of group F negative control. It maintained almost a straight line graphically (Figure 1).

Group of Mice	Mean WBC on day 0 after infection and before treatment	Treatment with Antioxidant vitamins (AV.)	Mean WBC on day 2 during treatment	Mean WBC on day 4 after treatment	Mean WBC on day 6 after treatment
A	16,080	Av.-A	13,420	11,800	11,220
B	17,160	Av.-C	12,460	10,560	10,200
C	16,100	Av.-E	12,140	10,660	10,660
D	15,880	Av.-A,C and E	10,660	9,740	9,940
E	17,100	No treatment (Infected)	23,200	Mice-dead	-
F	10,260 (not infected)	No treatment	10,560	10,120	9,500

Table 2: Mean White Blood Counts (WBC/mm³) in mice. A highly significant decrease ($F = 40.94, P < 0.0001$) in the mean WBC throughout the study.

Discussion

During the study, Antioxidant vitamin C was found to be very painful to mice when administered by intraperitoneal route. The mice reacted aggressively and needed to be done with great skill.

Infect, clearance of parasitaemia could be attributed to activation of phagocytic cells by antioxidant vitamins for possible phagocytosis [18]. Antioxidant vitamins stop proliferation of free radicals by inactivation of catalysts (iron and copper) released during malarial episode from the ruptured red blood cells [19]. Antioxidant vitamins are free-radical scavengers, and they act as anti-toxins [20]. Antioxidant vitamins are analogous to passive immunization.

Thumham., *et al.* [21] worked on the status of Vitamin A in malaria cases. It was recorded that patients with vitamin A deficiency were greatly susceptible to malarial attacks.

The treatment of malaria in mice with the use of antioxidant vitamins was found very effective. It took care of malarial infections; served as nutritional supplementary therapy by appreciating or raising the packed cell volumes after treatment. There was no recrudescence of infection after treatment. Combination of Antioxidant vitamins ACE enhance mostly the effectiveness of treatment, than the management with each antioxidant vitamin.

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sode from the ruptured red blood cells [19]. Antioxidant vitamins are free-radical scavengers, and they act as anti-toxins [20]. Antioxidant vitamins are analogous to passive immunization.

The study showed that Antioxidant vitamins regimen has proved to be an alternative to the use of synthetic antimalarial. Chloroquine, the most commonly used drug, has become resistant to *Plasmodium falciparum* [22-24]. The imminent failure of chloroquine is a threat to effective treatment, regardless of some serious side effects which include pruritus, cardiovascular toxicity, ocular toxicity which has irreversible adverse effect on the optic nerves [25-29]. With the emergence of Chloroquine Resistant Plasmodium falciparum (CRPF) malaria, treatment has become a very big public health problem. This has necessitated to various methods of approach for treatments, orthomolecular principle with free radical concept was found quite effective in malaria management. Application of this concept could end an unending search for new antimalarial drugs [30,31].

Conclusion

The prevalence of *Plasmodium* infection and continual spread of chloroquine resistant strains should necessitate taking a step into orthomolecular approach with free-radical concept for the management of Plasmodium infection. Administration of antioxidant vitamins take care of malarial infections, serve as nutritional supplementary therapy and also boost the immune system for proper health maintenance.

Antioxidant therapy becomes imperative as it modulates the effect of reactive oxygen species or free-radicals in malarial patients. It serve as anti-toxin or free-radical scavenger. When in cooperated in home management of malarial infection, it promotes nutritional status protect immune system and enhance life-style by preventing incessant malarial attacks.

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